SEED QUALITY OF ISOGENIC ENDOSPERM MUTANTS IN MAIZE

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Dedicated To

My Beloved Parents

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ABSTRACTS

Five studies were conducted based on ten near-isogenic corn hybrids converted to four endosperm genotypes -- +, *su*, *bt* and *sh2*.

Five different types of germination tests were conducted, including accelerated aging. Among the four endosperm genotypes, seed with wild type endosperm always had the best viability, followed in turn by *su*, *bt* and *sh2* hybrids.

Electrolyte leakage caused by accelerated aging (AA) was highly correlated with the deterioration of viability caused by AA. Seeds with poor germination ability suffered more from AA, indicating that seeds with good germination ability will have better storability. The accelerated aging, therefore, should be useful for predicting viability loss in seed storage.

Pericarp thickness was influenced greatly by endosperm mutant genes. Pericarp thickness of *sh2* hybrids at 36 days after pollination (DAP) were significantly greater than those at 18 DAP, while thinning trend of pericarp thickness was observed for wild type hybrids. There was no significant difference for *bt* and *su* hybrids from 18 to 36 DAP. A highly significant correlation was observed between seed weight and the difference of pericarp thickness harvested at 18 and 36 DAP. The data were interpreted as evidence for the effects of inner (endosperm) pressure on

pericarp distension.

The comparisons of eating quality among *su, bt* and *sh2* endosperm mutants showed in general, that *su* had the worst and *sh2* had the best eating quality considering sweetness and flavor, although the difference between *bt* and *sh2* was not significant for many hybrids. The extensive genotypic variability for these eating qualities among the *bt* hybrids suggests that the allelic variation at loci other than *bt* is probably involved.

Six germination-related characters were evaluated for *bt* and *sh2* hybrids. These were seed weight, pericarp thickness, bubble volume, seed density, leachate conductivity and sweetness. In general, *sh2* seed was sweeter with lower seed weight and density, and higher pericarp thickness, leachate conductivity and bubble volume (between endosperm and pericarp in mature seeds). These differences could be largely attributed to the efficiency of *bt* and *sh2* genes in hindering the conversion of sucrose to starch.

A method of measuring bubble volume through the change of soapy water volume was developed. The hypotheses were proved that bubble volume was determined by both shrinkage of endosperm and pericarp thickness, and thick pericarp affects germination rates of supersweet corn adversely through its effect on the formation of a large bubble volume. Bubble volume was observed to cause severe imbibition damage even with intact pericarps.

The correlation between seed weight and germination was positive and significant at 5% level. The correlations of germination with pericarp thickness, bubble volume, seed density and conductivity were highly significant, as were the correlations among these four characters. However, germination was not significantly corrected with sweetness (organoleptic).

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CHAPTER 1

INTRODUCTION

Sweet corn (*Zea mays L.*) cultivars grown in the United States have normally been homozygous for the recessive endosperm mutant allele *sugary-1*[1] (*su*). The *su* mutation reduces levels of a starch-debranching enzyme, creating a highly branched and soluble starch known as phytoglycogen (Pan and Nelson, 1984). The *su* endosperm has 8 to 10 times more phytoglycogen and twice as much sugar more than field corn in immature ears, 18 to 22 days after pollination (Creech, 1965).

Sweet corn hybrids homozygous for *su* have a major limitation, due to the relatively short period during which kernels are at peak quality. Ears of sweet corn (*su*) are characterized by a rapid loss of quality after harvest due to the conversion of sugar and phytoglycogen to starch. Sugar levels in kernels decreased 40 to 60 percent during the 24 hour period after harvest when stored at 25°C (Carey *et al.*, 1982). This decline in quality restricts shipment

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[1] In accordance with revised maize genetics nomenclature (Burnham *et al.*, 1975), the genes formerly called *su-1* and *bt-1* are now designated *su* and *bt*. Allelic designations, when known, follow the dash.

from fresh sweet corn growing regions to major marketing areas. Rapid changes in *su* ear quality also provide a very narrow harvest window to assure profits to the sweet corn processing industry (Mashall, 1987).

In the last 20 years endosperm gene mutations other than *su* have been used to develop new commercial sweet corn, due to the problems related to *the su* gene (Boyer and Shannon, 1983). There are at least 13 distinct single gene endosperm mutations in *Zea mays* L. which produce qualititative and quantitative differences in maize kernel carbohydrate metabolism (Hoisington et al., 1988).

The most common mutant gene utilized in American hybrids is *shrunken-2* (*sh2*), originally known as "Xtrasweet" corn and now generally described as supersweet corn. Laughnan (1953) reported that almost 20% of the dry matter of the shrunken kernels was sugar, a 10-fold increase over field corn. Most of the sugar consisted sucrose. A corresponding decrease in starch was observed. This gene is known to reduce activity of ADP-glucose pyrophosphorylase, an enzyme that is important in the conversion of sucrose to the substrates for starch synthesis. This leads to increased sugar content and reduced seed viability and storage longevity. Supersweet corn also has superior post-

harvest qualities. These include high sugar retention (Garwood *et al.*, 1976), reduced moisture loss (Wann *et al.* 1971), and delayed post-harvest conversion of sucrose to starch without the refrigeration requirement of standard cultivars (Boyer and Shannon, 1983).

The tropical supersweet corns bred in Hawaii are based on an unrelated gene, *brittle-1* (*bt*) (Brewbaker, 1977). The *brittle-1* (*bt*) locus of maize was identified in 1926 by mutations that severely decreased the amount of starch deposition in the endosperm (Mangelsdorf, 1926; Wentz, 1926). Although *bt* mutant kernels are low in a starch granule-bound phospho-oligosaccharide synthase, the primary function of the *bt* gene products is not known (Pan and Nelson, 1985). Sullivan *et al.* (1991) proposed that the maize *bt* locus encodes an amyloplast membrane metabolite translocator protein, based on the protein sequence deduced from a cDNA clone. The *bt* gene appears to affect membrane transport of sugars and greatly increase seed sugars, reduce seed viability and storage longevity.

Breeding stocks have been developed in Hawaii (Brewbaker, 1974) that permit comparison of the *shrunken-2* and *brittle-1* genes in Near-Isogenic Line (NIL) backgrounds, together with sugary mutant gene *sugary-1* (*su*) and the

normal wild-type. The NIL backgrounds include tropical and temperate inbreds. The combination of mutant and background genes is important in breeding tropical supersweet corns that can be grown with minimum pesticide application.

Objectives of this thesis were:

1) To compare the effects of endosperm mutant genes on viability loss and seed leachate conductivity.

2) To study the relative length of storability of different endosperm mutants following accelerated aging of seeds.

3) To study the effects of endosperm mutant genes and maturity on pericarp thickness, and test the hypothesis that the inner pressure can largely influence pericarp thickness during kernel development.

4) To compare the eating quality of sweet corn (*su*) and supersweet corn (*bt* and *sh2*) mutant genes.

5) To compare the effects of endosperm mutant genes *bt* and *sh2* on six characters that relate to germination rates.

6) To study correlations among six measured characters and between these characters and germination rates.

7) To quantify the bubble volume of kernels and test the hypotheses that bubble volume determined by shrinkage of endosperm and pericarp thickness, and pericarps affect germination of supersweet corn through their effects on the formation of bubble volume.

CHAPTER 2

LITERATURE REVIEW

2.1 Seed viability and accelerated aging

2.1.1 Viability

The fresh market sweet corn industry in the United States has shown a shift in recent years from traditional hybrids with *su* endosperm to hybrids homozygous for the *sh2* endosperm mutation. Germination and seedling vigor of high-sugar *sh2* seeds, however, can be considerably less than normal and *su* seeds. This is especially true when soil temperatures are lower than 15°C (Andrew, 1982; Kulik and Schoen, 1982). Slow germination, poor stands and reduced seedling vigor often make the high-sugar cultivars unacceptable for commercial production (Hannah and Cantliffe, 1977). Wann (1980) found early seedling growth to be greater in the *su* hybrids of 'Iobelle' than in its *sh2* counterpart hybrid 'Florida Staysweet'.

Styer *et al.* (1980) compared germination and seedling vigor of normal, *su, sh2* and *bt* kernels harvested at different times after pollination (not on the same genetic background). They reported that germination and seedling vigor (germination rate, radicle length, fresh and dry weight) of *sh2* variety was significantly lower in both laboratory and field tests than *su, bt* and normal. The latter three genotypes were nearly equal in seedling vigor.

Several investigators demonstrated that soil-borne fungi were the most important cause of maize pre- and post-emergence damping-off under stress condition. *Fusarium moniliforme* (Styer *et al.*, 1983; Kulik *et al.*, 1982) and *Pythium spp.* (Callan *et al.* 1990; Harper *et al.*, 1955; Hoppe, 1950) are reported to be particularly important. Brewbaker (1992) reported that in general, temperate inbreds (and notably those with genes such as *opaque-2* or *shrunken-2*) are highly susceptible to *Fusarium spp.* as a seedling or kernel rot in Hawaii.

The higher sugar content of the *sh2* kernels was associated with an increase in rot and pathogens during germination (Berger and Wolf, 1984). Leakage of nutrients from corn kernels can provide essential nutritive substances for fungi to develop on and around the seed (Schroth and Cook, 1964).

Waters *et al.* (1983) reported that germination in cold tests conducted in sterile sand and on rolled towels at 10°C for 6 days was highly correlated with sweet corn (*su*) field emergence, but standard lab germination tests were not. Cold soil test at 10°C for 7 days was superior for prediction of the field emergence of a wide range of field corn inbred lines ($r = 0.74$) (Martin *et al.*, 1988) and for *sh2* supersweet corn ($r = 0.95$) (Parera *et al.*, 1995).

Moisture content of the soils played a very important role in soil cold tests, especially for poorer seed samples

(Nijenstein, 1985). Harper *et al.* (1955) observed a close correlation between soil cold test and soil moisture content, concluding that seed mortality was greatest when soil moisture content was high.

Conductivity is a measurement of seed electrolyte leakage. Significant correlation between seed conductivity and field emergence of both *su* and *sh2* endosperm has been reported (Waters and Blanchette, 1983; Tracy and Juvik, 1988). Seed leachate is comprised primarily of potassium, phosphate, sugars, amino acids, proteins, and various other electrolytes (McKersie and Stinson, 1980).

Conductivity was affected by length of imbibition, inbred background, and endosperm-type main effects and interactions of these effects (Schmidt and Tracy, 1989). Exposure to chilling temperatures must be relatively long before cells of sensitive plants were injured. In general, the severity of injury of sensitive plant tissues increased as temperature was lowered, or as the exposure was extended at any chilling temperature (Lyons, 1973). The temperature in the first 24 hours after planting was critical to the performance of *sh2* seed (Tracy, 1989). Temperature effects on seed imbibition and leakage were mediated by solution viscosity and membrane permeability (Murphy *et al.*, 1982). Studies in which permeability change are measured by solute leakage have provided some direct evidence for increased membrane permeability in response to chilling (Lyons, 1973).

Maximum field emergence of *sh2* hybrids was obtained for seeds harvested before dry-down, at 36 days after pollination (Styer *et al.*, 1980).

Interactions between endosperm genes and germplasm backgrounds can significantly affect seed quality (Rowe and Garwood 1978; Soberalske and Andrew 1978, 1980). Isogenic inbred lines were a prerequisite for evaluating the contribution of genes (Brewbaker, 1974).

2.1.2 Accelerated aging

The seed storability of supersweet corn with endosperm genes *bt* or *sh2* was much shorter than that of field corn or sweet corn (*su*) (J. L. Brewbaker, personal communication). Delouche and Baskin (1973) reported on accelerated aging (AA) for various crops. Maize seeds were subjected to AA at 40°C and 100% RH for 5 days, showing that the test was useful in predicting the relative storability of maize seed lots. Those seeds that deteriorated rapidly under conditions of AA also tended to perform poorly in long-term open storage.

Many studies indicate that the release or leakage of solutes during imbibition can be broadly correlated with aging (Chin and Schoolcraft, 1968; Powell and Matthews, 1978; Parrish and Leopold, 1978). This leakage was attributed to a loss of membrane integrity. Although the exact mechanism causing the membrane alteration in seed

aging is not known, speculation surrounds the role of peroxidation and autocatalytic oxidation of unsaturated fatty acids (Bewley, 1986). The principle of the conductivity test assumes that seed deterioration is manifested by loss of membrane integrity. If electrolyte leakage is, in fact, highly correlated with deterioration, this test should have wide applicability for predicting seed storability (Roos, 1989). Schoettle and Leopold (1984) reported that solute leakage from imbibed soybean cotyledons increased linearly with AA. A major contribution to the A_{260}-detected solutes leaking from soybeans after AA was from cells which had experienced massive membrane damage.

Rapid water uptake during the initial phase of imbibition can negatively affect germination (Powell and Mathews, 1978; Chern and Sung, 1991). Supersweet corn (*sh2* gene) was prone to imbibition damage due to its rapid influx of water (Simon, 1978; Wann, 1986). The elevated levels of sugars increased osmotic potential, and the bubble space between pericarp and aleurone layers allowed the pericarp to be broken easily, facilitating water movement into and out of the seed. When rate of water absorption was reduced during the early stage of imbibition, tissues developed in an organized manner. This allowed sufficient time for membrane rearrangement, and the imbibition damage was significantly reduced (Parera and Cantliffe, 1991; Chern *et al.*, 1991).

The accelerated aging (AA) test has become a fairly accepted test for seed vigor. Kulik and Schoen (1982) reported that the AA test was correlated with field emergence of *sugary-1* (*su*) sweet corn within genotypes. Wilson and Trawatha (1991) found that the soil cold test, accelerated aging, and mean leachate conductance were highly correlated with final stand of a *sh2* hybrid (median r = 0.87, 0.85 and -0.88, respectively).

2.2 Food Quality

2.2.1 Pericarp thickness

Pericarp thickness is implicated in several important roles relating to kernel quality, including tenderness of food corns (Ito and Brewbaker, 1981), quality of popcorn (Richardson, 1965), and resistance to pathogens (Wolf *et al.* 1952). It also significantly influences field emergence of supersweet corn via the formation of bubble volume (see Chapter 6).

Helm and Zuber (1970) compared nine endosperm mutants with their normal versions in inbred backgrounds B37 and Oh43. They reported that the pericarp, being maternal tissue, was not greatly influenced by the genotype of endosperm when averaged over all mutants. However, in the B37 series the pericarp thickness for the *sh2* mutant significantly exceeded its normal sibbed kernels (no Oh43*sh2* was in this study). They also found that for pericarp

thickness there was no metaxenia effect (1972a), no reciprocal effect (1972b), no effect of harvest date once physiological maturity has been reached (1970) and little environmental effect (1969).

Martin *et al.* (1979) found no significant difference in pericarp thickness between normal and *opaque-2* kernels segregating on the same ears. Juvik (1992, unpublished) reported that in two sets of isolines isogenic for *Su, su, se* and *sh2,* the force needed to puncture the pericarp was greatest in *sh2* and the least in *Su* and *se*. Ito (1980, unpublished) reported that 15 mutants backcrossed to CM104 were evaluated for pericarp thickness. Generally, all mutants were of similar pericarp thickness except *sh2* mutant, which had a significantly thicker pericarp thickness.

Ito and Brewbaker (1991) concluded that at least three different morphological changes were involved in pericarp thickness polymorphism: 1) the number of pericarp cell layers, which can range from two to > 20, lower values being found only in teosinte (Tracy *et al.*, 1987), 2) differential thickening of the pericarp on germinal and abgerminal surfaces, and 3) the thickening of individual pericarp cells.

Randolph (1936) reported the expanding endosperm exerted pressure on the pericarp and ultimately affected its thickness at maturity, a conclusion that has not been

verified in later studies.

Wolf *et al.* (1952) reported that pericarp thickness of dent corn was affected by location of the pericarp on the kernel. Pericarps were much thicker at the base of the kernel than in the central and upper region. They were also appreciably thicker over the back of abgerminal surface than over the germ. The thinnest pericarp was over the dent cap region. The variation in thickness was believed due primarily to differences in compression over different parts of the kernel rather than to differences in the number of cell layers. Haddad (1931) studied the relationship between inbred lines and their F_1 hybrids. He found the number of cell layers of the pericarp was the same in the hybrid as in the parental inbreds. However, heterosis for thin pericarp was observed. The differences in thickness was due to reduced cell wall thickness and not to changes in cell numbers.

Richardson (1960) studied crown portions of popcorn pericarp. He found that stage of maturity affected pericarp thickness of popcorn. Pericarp thickness underwent a gradual decrease with the minimum thickness at 32% of kernel moisture, i.e., physiological maturity. He suggested that the early decrease in pericarp probably resulted from stretching caused by the enlargement of the endosperm, in addition to the loss of water and decreased succulence of the pericarp. When the lowest point of the curve had been

reached, a gradual increase in pericarp thickness began and continued until about 24 days after physiological maturity. Although no lignin analysis was reported, he attributed this occurrence to the lignification of pericarp tissue.

Culpepper *et al.* (1924) found a decreasing pericarp thickness and increasing toughness 15 days after pollination for sweet corn. Azanza and Juvik (1992) reported that a 25% decrease in tenderness occurred between the harvest maturities from 18 to 22 days after pollination for different endosperm mutants.

Helm and Zuber (1970) studied dent corn inbred lines and found no significant difference in mature pericarp thickness from excised pericarps when the ears were harvested at 15% or 30% moisture content. Their results were different from those of Richardson (1960), who found significant differences between pericarp thickness at various harvest stages after physiological maturity. However, Richardson measured crown tissues whereas Helm and Zuber measured thickness around the side of the kernels and discarded the crown pericarp tissue.

Tracy *et al.* (1987) found that the endosperm combination *Su su2* had significantly thicker pericarp (48 μm) than any of nine other endosperm types observed. Endosperm combination *su su2* had the thinnest pericarp, 38.7 μm, but was not significantly different from *Su sh2* (38.7 μm) and *su* (39.1 μm). They concluded that *Su su2* endosperm

may actually expand less then most other endosperm types, exerting less pressure on the pericarp, and therefore, resulting in a thicker pericarp (Tracy *et al.*, 1988).

Studies have indicated that the quantity of the pericarp increased with advancing maturity, despite the thinning trend of the pericarp. Groszmann and Sprague (1948) measured the weights of the pericarps periodically for a period of 52 days after pollination and found that pericarp weights increased somewhat rapidly during the late stages of development. Barton (1954) found that there is greater percent pericarp by weight as the kernels approached maturity.

The inheritance of pericarp thickness also has been studied and found to be controlled by oligogenes with varying degrees of dominance for thin pericarp (Richardson, 1965; Helm and Zuber, 1972; Ho *et al.*, 1975; Ito and Brewbaker, 1991).

2.2.2 Sensory evaluation

The fresh market sweet corn industry in the United States has shown a shift in recent years from traditional hybrids with *sugary-1* (*su*) endosperm to hybrids homozygous for the *shrunken-2* (*sh2*) endosperm mutation.

The traditional sweet corn (*su*) had greatly increased levels of water-soluble polysaccharides (primarily phytoglycogen) and twice the sugar content of field corn

(Wann *et al.*, 1971). Phytoglycogen is important for sweet corn quality, since characteristics of texture and creaminess are affected by the water soluble fraction and the ratio of soluble to insoluble polysaccharides (Culpepper and Magoon, 1924).

Supersweet corn with *sh2* mutant had two to three times more sucrose at 20 days after pollination than *su* kernels (Creech, 1956), and retained higher sugar levels for longer periods after harvest (Garwood *et al.*, 1976). They have been generally preferred over *su* sweet corn in consumer taste tests (Evensen and Boyer, 1986). The *sh2* mutant had only 10%-20% of the phytoglycogen content observed in *su* sweet corn (Dickinson *et al.*, 1983).

Supersweet corn based on *brittle-1* (*bt*) gene was similar to *sh2* supersweet corn. It had higher sucrose content and superior postharvest qualities such as sugar retention compared to traditional sweet corn (Brewbaker, 1977; Banafunzi, 1974).

Flavor, sweetness and tenderness are the most important factors determining food quality of sweet and supersweet corn. Although sweetness was found to be the principal component of flavor, kernel creaminess and moisture as well as the ratio of insoluble to soluble components of the endosperm were significant quality parameters (Boyer and Shannon, 1983).

Correlation coefficients between sugar content and

flavor as measured by taste panel scores (Winter, 1955) were higher when the corn was relatively lower in sugar. When the sugar level was high, other variables became more important in determining the relative palatability of the sample tested.

Juvik (1992) reported extensive variability among 24 commercial *sh2* hybrids of sweet corn. He suggested that the genotypic variability among *sh2* hybrids could indicate allelic variation at loci other than *sh2,* influencing sucrose and total sugar levels in fresh harvested sweet corn. Sensory evaluation of the same samples suggested that sweetness, starchy flavor, crispness and tenderness were the key sensory attributes correlated with overall like. He also found that sweetness was strongly correlated with sweet corn flavor, total sugar and sucrose.

Tenderness mainly consists of pericarp thickness and endosperm texture. The bite test was the most effective and convenient method to determine the tenderness of supersweet corn (Brewbaker, 1977). A significant correlation (r^2 =0.96) was found between average bite-test scores and immature pericarp thickness, but correlations based on individual ears were low (r=0.24). Bite-test scores were subject to high error variability (CV=25%) as opposed to pericarp micrometry (CV=12%) (Ito and Brewbaker, 1981).

2.3 Characters related to germination of supersweet corn

The incorporation of the mutant *sh2* and *bt* genes in sweet corn has greatly improved sweet corn eating quality (Garwood *et al.*, 1976; Brewbaker, 1977). Commercial acceptance and widespread use of *sh2* and *bt* has been hindered, however, by inferior quality of growers' seed, reduced emergence, poor seedling vigor and erratic stand uniformity, especially in cold soil (less than 15°C) (Andrew, 1982; Kulik and Schoen, 1982; Styer *et al.*, 1980).

Many proposals have been made to explain these phenomena. Poor vigor of *sh2* sweet corn seed has been associated with dysfunctional aleurone and a shortened scutellum-endosperm interface (Harris and DeMason, 1989). It is also attributed to damage associated with mechanical harvesting and rapid drying of hybrid seed (Marshall, 1987). Commercial practice is to harvest *sh2* seed soon after physiological maturity with great care in drying and shelling (Brewbaker, personnel communication). The poor vigor of *sh2* seed might arise from inadequate maturity (Wilson and Trawatha, 1991) and lack of desiccation tolerance (Wilson, 1992). Reduced content of starch and phytoglycogen in *sh2* kernels might not provide sufficient carbohydrate reserves for optimal rates of emergence and growth of seedlings (Wann, 1980).

Elevated levels of sugars increase osmotic potential and lead to membrane damage from the rapid influx of water

during imbibition (Simon, 1978). Rapid water uptake during the initial phase of imbibition could negatively affect germination (Powell and Mathews, 1978; Chern and Sung, 1991).

Bubble space is a very common phenomenon in supersweet corn seed. It is the separation of endosperm from pericarp, creating a vacuole or bubble. The dramatically reduced starch content of *sh2* kernels results in a severely collapsed endosperm and large bubble space between the pericarp and aleurone layers, which allows the pericarp to be broken easily upon handling. This facilitates water movement into and out of the seed (Wann, 1986), and can accelerate movement of the water-soluble fractions out of the seeds (Douglass *et al.*, 1993). *Fusarium moniliforme* penetrated *sh2* corn kernels via small cracks in the pericarp, where the fungus localized in the bubble space between the pericarp and aleurone layer, and eventually moved into the endosperm and embryo (Styer and Cantliffe, 1983). Broken pericarp was associated with decreased germination, emergence, and seedling vigor (Koehler, 1957).

Pericarp thickness of sweet and supersweet corn played a key role in both the quality of grower's seed and the fresh or processed product consumed by the public. Thicker pericarp might also better resist internal pressure during kernel development and prevent splitting and exposure of the seed to pathogens (Helm and Zuber, 1969).

Conductivity is a useful measure of electrolyte leakage. Significant correlation between seed conductivity and field emergence of both *su* and *sh2* genotypes has been reported (Waters and Blanchette, 1983; Tracy and Juvik, 1988). Solute leakage during germination reduces metabolic energy available for embryo growth and might provide substrates for the growth of pathogenic microorganisms (Styer and Cantliffe, 1984).

Kernel density is usually expressed as specific gravity in comparison with water (specific gravity of 1.0). Measuring density of corn in water would give a direct reading, but corn kernels tenaciously trap air bubbles when placed in water, although not in ethanol or toluene. Many researchers used ethanol displacement as a useful, convenient way to estimate kernel density (Watson, 1987). The surface tension of ethyl alcohol (0.022 N/m), toluene (0.028 N/m) and soapy water (0.025 N/m) are much lower than that of water (0.073 N/m) (Green, 1984). All density tests are affected by moisture content (Nelson, 1980). The density of an average dent corn at 12% moisture content is 1.2, of flour corn 1.1, and of popcorn or flint corn up to 1.3 (Watson, 1987).

Wann (1980) suggested that the nutrient reserve for the endosperm was critical and that increased seed weight should aid in germination. Andrew (1982) reported that among *sh2* inbreds used as seed parents, seed weight was not related to

germination rates or seedling vigor, but that within a seed parent, the largest seeds resulted in improved germination and seedling vigor. Eleven cycles of mass selection in a population of *sh2* corn significantly increased field emergence and seed test weight, and a highly significant correlation (r^2 = 74%**) was found between them (Bell *et al.*, 1983).

Douglass *et al.* (1993) reported that there was a highly significant negative correlation (r^2=0.55) between the kernel sugar content and sweet corn (*su*, *se* and *sh2*) field emergence in cold soil.

CHAPTER 3

MATERIALS AND METHODS

3.1 Seed viability and accelerated aging

3.1.1 Viability

The effects of endosperm mutant genes (*bt, sh2, su* and normal wild type) on viability under cold and *Fusarium* stress were evaluated on near-isogenic backgrounds. Seed leachate conductivity and various germination tests were used in this study.

Field emergence test: Fifty seeds were planted in a one-row plot, using a split-split plot design with two fungicide treatments (treated vs. untreated) as main plots, the four endosperm types as subplots, and genotypes as sub-subplots. Three replications were used. Final stand counts were recorded at the six-leaf stage. The fungicide used in this trial was 'Captan'. This experiment was conducted at the Waimanalo Research Station of the University of Hawaii on January 10, 1994.

Warm germination tests: A slightly modified version of the standard towel germination test (Anonymous 1983) was used. Three replicates of 25 seeds of each genotype were rolled in moist paper towels. The rolled paper towels then were placed upright in tinplated containers for 6 days germination at a room temperature of 25°C. Only emerged seedlings with at least 3 mm of radicle and coleoptile were

considered as germinated. The rest, including the severely abnormal seedlings, were not counted.

Cold soil tests: This method is a modification of the cold soil test procedure described by Woodstock (1976). Three replicates of 25 seeds of each genotypes were placed on the middle-upper portion of the moist germination papers and covered with sand/soil (2:1) mixture, then rolled. The rolled paper towels were put in a plastic bag, sealed and placed upright in a LAB-LINE incubator for 7 days at 10°C in the dark, then transferred to 25°C for 6 days. The soil was collected from the corn field at the Waimanalo Research Station of the University of Hawaii and allowed to stabilize for two weeks before use. Failure to allow stabilization might cause erratic test results (Anonymous, 1984). The soil is a Vertic Haplustoll with pH 6.0 derived from coral and lava intrusions (Brewbaker, 1985). *Fusarium spp.* are epidemic in Waimanalo soils where corn has been grown and stubble plowed down continuously over 20 years (J. L. Brewbaker, personal communication).

Cold test (without soil): The procedure for the cold paper-towel test was the same as for the cold soil test above except that no soil was applied.

3.1.2 Seed leachate electrolyte conductivity (SLEC)

SLEC was measured by using a modified version of the method developed by Water and Blanchette (1983). Vials

containing 10 seeds of each sample were filled with 20 ml deionized water and placed at room temperature of about 25°C for 24 hours. The conductance reading was taken by a YSI model 32 conductance meter. The SLEC test was conducted under only room temperature, since it was found that the conductivity of each sample under cold temperature (10°C) for 24 hours was lower than the one under room temperature. It is difficult to evaluate the effect of low temperature on germination rate through the effect of low temperature on conductivity. A similar phenomenon has been reported on several species by Leopold (1980) and Murphy *et al.* (1982), who concluded that temperature effects on seed imbibition and leakage were mediated by water viscosity and membranes.

Conductivity was determined by multiplying the measured solution conductance by the cell constant K. In this study, the conductivity cell used was YSI 3417 with a S.I. cell constant of K = 100/m. In S.I. units, 1 siemens equals 1 mho, for an observed conductance of 100 microsiemens. Conductivity (k) was expressed as k = 100μS x 100/m = 10 milli S/m (milli Siemens/meter) (Anonymous, 1989).

3.1.3 Accelerated aging (AA)

All seeds were harvested at physiological maturity (36 days after pollination), and then dried under forced air to a moisture content (MC) of about 13 percent. The seeds were then subjected to 97-100% relative humidity (RH) at 46°C for

72 hours prior to germination tests (Wilson and Trawatha, 1991). Each genotype of seeds was placed in a glass bottle that was placed in a tray with water. The tray was then placed into a thick plastic bag to retain moisture, and the bag put into an oven incubator. Temperature was kept at 46°C and the relative humidity was maintained at about 97-100%.

The two AA germination tests are AA without drying (W/D) and AA after drying under room temperature (25°C). Since the higher RH% of AA may relieve the imbibition damage of supersweet corn seeds, the seeds after the AA treatment were separated into two sets, AA without drying (W/D) and AA after drying (A/D). The W/D seeds were directly used after AA. The A/D seeds were dried again after AA for two days in 30°C in a NAPCO mechanical convection oven (Model 603). AA conductivity tests, like normal conductivity tests, were only conducted at room temperature.

Three replicates of 25 seeds of each variety were used in a split-split plot design with the two sets (AA without drying vs. AA with drying) as main plots, the four endosperm types as subplots, and genotypes as sub-subplots. Both standard lab and cold paper-towel germination tests, as well as conductivity tests (as mentioned in 3.1) were used to evaluate viability loss and membrane damage.

3.2 Food Quality

3.2.1 Pericarp thickness.

Pericarp thickness was measured from germinal and abgerminal sides at a medial position on ten seeds per sample, generally following methods of Helm and Zuber (1972) as modified by Ito and Brewbaker (1981). Seeds harvested at both 18 and 36 days after pollination were used in this experiment.

Ears of each variety were air-dried to about 15% moisture. shelled and sampled. The kernels were soaked in tap water for 20 hours at room temperature or in the reefer for a longer time. The crown and tip caps of ten kernels per sample were removed with a razor blade, and the pericarp slit along the edge of the kernel and peeled off with tweezers.

The result was a rectangular strip of excised pericarp with a germinal and an abgerminal face. The excised pericarps were placed overnight in a solution of 2 water : 1 glycerin (by volume) and evacuated in a vacuum desiccator. After evacuation, they were allowed to stand for 20 hours at room temperature. They were blotted dry between tissue papers, the papers were put in a book seperatedly and put weight on it, then equilibrated at room humidity (60%) overnight before measurement with an Ames no. 56212 micrometer (Ames, Inc., Waltham, Mass). Remaining aleurone tissue, if present, was removed by scraping.

3.2.2 Sensory evaluation

Sensory panel evaluations on *bt*, *sh2* and *su* sweet corn for flavor, sweetness, tenderness and crispness were conducted on the same ears at 18 days after pollination. Samples were tested immediately after harvest. The varieties could not always be tested on the same day due to differences in maturity.

Each of three ears for each variety were tasted by three experienced panelists under both fresh and steam-cooked conditions. The evaluation tests were conducted twice. The average data from each of the three ears were considered as replications. A 1 to 9 hedonic scale was used in this experiment, in which 1 represents the best and 9 represents the worst.

3.3 Characters related to germination of supersweet corn

3.3.1 Quantifying bubble volume of supersweet corn seeds

Three samples of ten seeds of each variety with *bt* or *sh2* gene were immersed into 5 ml soapy water (including the volume of a 5 g balance weight) in a 10 ml graduated cylinder. The cylinder was shaken to get rid of some bubbles. The volume was recorded and named V1. These ten seeds should have no cracks on their pericarps.

Soapy water with very low surface tension and a small friction coefficient was chosen in this experiment, in order

to prevent the formation of small air bubbles on the surface of the shrunken seeds and to facilitate the replacement of the air in bubble space for the liquid. The friction coefficient of soapy water with corn hilar orifice was not possible to obtain, but it should be much lower than that of ethanol which has often been used to measure seed density. At 20°C, the surface tension for water is 0.073 N/m and soapy water (no information about what soap and concentration has been mentioned) is 0.025 N/m (Wilson, 1994). A 5-gram weight on a thin string was used to push seeds into the soapy water, since some of the supersweet seeds tended to float on the surface. The dishwashing liquid "Ivory" bought from the supermarket was used and the concentration of the soapy water was 1.5 ml:250 ml. At this concentration, the foam which formed during evacuation could be kept in the cylinder.

The cylinder containing the seeds was then put into a 1 liter filtering flask that could be evacuated with a tap water vacuum. During about 3 minutes of evacuation, most of the air in the bubble space was sucked out and replaced by soapy water. The volume of soapy water was then recorded and named V2. The bubble volume of each variety was the reduced volume and calculated by V1 minus V2 and converted to units of ml/100 seeds. The procedure of measuring bubble volume was showed in figure 3.1.

Figure 3.1 The procedure of measuring bubble volume

3.3.2 Measuring seed density

Three samples of 25 seeds of each *bt* or *sh2* variety were weighed and then immersed in 17 ml soapy water (including the volume of a 10 g balance weight) in a 25 ml graduated cylinder. This was shaken to get rid of some bubbles, and the increased volume (V1) was recorded. V1 was the magnitude of 17 ml soapy water and the volume of 25 seeds, thus V1 minus 17 ml equalled the volume of 25 seeds. Seed density equalled seed weight divided by volume in units of gram/ml. The soapy water used here was the same as that used in 3.3.2.

3.4 Materials

Reasonably isogenic lines of four endosperm mutant genes *(bt, sh2, su* and wild type) were available for this study in four inbred lines and one composite. The near isogenic line (NIL) series of Hi27 and Hi38 as well as HS were converted by Dr. J.L. Brewbaker (Brewbaker, 1974). Hi27 series was converted from an Indian flint inbred CM104. Hi38 series was converted from AA8 which is a *su* inbred derived from a *su* variety "Hawaiian Sugar". HS series was composites converted from the *su* variety "Hawaiian Sugar". In fact, HS*bt* was a very successful composite variety named "Hawaiian Supersweet #9" released in 1977 (Brewbaker, 1977), and has been improved for tenderness (Ito and Brewbaker,

1981). The *bt* gene used in Hawaiian bred materials was obtained from the Maize Genetics Cooperative in the linkage group a_2*btpr* of a red aleurone (*ARC*) genotype (Brewbaker, 1977). The NIL series of B37 and Oh43 were provided by Dr. David V. Glover of Purdue University. The *bt* allele used in their materials should be the same as the one used in Hawaiian bred materials, since it was the only one available at that time (personal communication, Dr. J.L. Brewbaker). B37 is an inbred line derived from *Iowa Stiff Stalk Synthetic* with notably thick pericarp.

A partial diallel mating design was conducted, in which the five NIL series were grouped by endosperm genotypes and the partial diallel crosses were made within each group. Since there was no inbred line of Hi38*sh2*, only 36 different hybrids were produced. Table 3.1 presents the combinations of these 36 hybrids.

All experiments in this research were planted through 1992-1994 at the Waimanalo Research Station of the University of Hawaii, located at sea level and 21° N latitude on the island of Oahu. The sweet and supersweet corn stage of optimum quality occurs between 68 and 75 days (18 days after pollination) at this location. Inbred lines from US mainland were prone to severe leaf rust, producing no yield or giving immature kernels only. To solve this problem, fungicide "Dithane N45" was sprayed on mainland lines two weeks after pollination.

Table 3.1 The combinations of the 36 hybrids

Hybrids	
Hi27+ X B37+	Hi27su X B37su
Hi27+ X Oh43+	Hi27su X Oh43su
Hi27+ X HS+	Hi27su X HSsu
Oh43+ X B37+	Oh43su X B37su
HS+ X B37+	HSsu X B37su
HS+ X Oh43+	HSsu X Oh43su
Hi27+ X Hi38+	Hi27su X Hi38su
Hi38+ X B37+	Hi38su X B37su
Hi38+ X Oh43+	Hi38su X Oh43su
Hi38+ X HS+	Hi38su X HSsu
Hi27bt X B37bt	Hi27sh2 X B37sh2
Hi27bt X Oh43bt	Hi27sh2 X Oh43sh2
Oh43bt X B37bt	Hi27sh2 X HSsh2
HSbt X Hi27bt	Oh43sh2 X B37sh2
HSbt X B37bt	HSsh2 X B37sh2
HSbt X Oh43bt	HSsh2 X Oh43sh2
Hi38bt X Hi27bt	
Hi38bt X B37bt	
Hi38bt X Oh43bt	
Hi38bt X HSbt	

Simple linear correlation analyses were used and estimated by the coefficient of determination (r^2) in this study. It is the square of the correlation coefficient and is usually expressed as a percentage. All statistical analyses referred to the books "Experimental design on a spreadsheet" (Brewbaker, 1993), and "Biometry on a spreadsheet" (Brewbaker, 1994).

CHAPTER 4

SEED VIABILITY AND ACCELERATED AGING

Viability of all 36 hybrids was evaluated by five different germination tests. Three leachate conductivity tests were also conducted on seeds harvested at 36 days after pollination to corrected with viabilities. Methods for assessing germination rate and conductivity were described previously in Sections 3.1.1 and 3.1.2.

Germination data from the cold test, accelerated aging and conductivity tests were show to be highly correlated with field emergence of *su* and *sh2* genotypes by Waters *et al.* (1983) and Wilson *et al.* (1991). The seed accelerated aging (AA) test is used not only to predict the relative seed storability but also to test for seed viability and vigor. Imbibition damage of supersweet corn seed may be reduced by the high relative humidity of the AA treatment, therefore seed after the AA treatment was divided into two groups. The one directly used for germination and conductivity tests was called AA without drying (W/D), and the one which was dried again before germination and conductivity tests was called AA after drying (A/D). Methods for conducting the two types of AA tests were described in Section 3.1.3.

4.1 Viability

Germination rates: The data of the standard germination test in rolled towels at 25°C ranged widely from 25.3% to 98.7%, with an overall average of 81.1% (Appendix 1). The data of the cold test (in wet rolled paper towel at 10°C for 7 days and then transferred to 25°C) ranged from 20.0 % to 100%, with an overall average of 75.0% (Appendix 1). The data of the cold soil test (the same as cold test except adding soil) ranged from 6.7% to 90.7%, with an overall average of 46.0% (Appendix 4). The data of AA without drying germination rate ranged widely from 8% to 100%, with an overall average of 74.6% (Appendix 3), and of AA after drying germination rate ranged from 4% to 100%, with an overall average of 63.6% (Appendix 3).

The average germination rates of these five germination tests based on six isogenic hybrids (without Hi38) and ten (with Hi38) for +, *su*, *bt*, and *sh2* seeds are presented in Table 4.1. All of the five germination tests showed similar trends of average germination rates for the four endosperm mutants. Wild-type hybrids had the highest average germination rates, followed in turn by *su*, *bt* and *sh2* hybrids.

Among supersweet corn hybrids, Hi38*bt* X HS*bt* had the best germination rate in all of the germination tests. One exception was the low germination of Hi38*bt* X HS*bt* in the cold soil test. This appeared to be due to excessive

Table 4.1 Data of germination and conductivity tests

Hybrids	Germination rates					Conductivity (milli S/m)		
	Stand. Germ. Avg	Cold Germ. Avg	Cold Soil Avg	AA W/D Avg	AA A/D Avg	Stand. Avg	AA W/D Avg	AA A/D Avg
Hi27+ X B37+	96.0	100.0	76.7	96.0	94.7	10.6	12.0	11.4
Hi27+ X Oh43+	94.0	100.0	50.7	98.7	94.7	16.0	18.9	16.4
Hi27+ X HS+	94.0	96.7	70.0	97.3	98.7	8.0	9.1	8.4
Oh43+ X B37+	97.3	95.3	45.3	94.7	90.7	21.0	22.4	22.7
HS+ X B37+	96.7	95.3	90.7	97.3	97.3	12.6	15.3	13.4
HS+ X Oh43+	97.3	99.3	85.3	98.7	96.0	14.0	15.8	13.4
Hi27+ X Hi38+	98.7	98.0	68.0	93.3	96.0	7.7	9.7	9.1
Hi38+ X B37+	98.0	98.7	69.3	96.0	94.7	14.0	15.3	13.7
Hi38+ X Oh43+	98.7	99.3	64.0	100.0	97.3	12.9	14.2	14.1
Hi38+ X HS+	98.7	98.7	89.3	100.0	100.0	8.0	8.6	9.1
Hi27su X B37su	82.7	87.3	45.3	86.7	70.7	17.1	18.8	19.8
Hi27su X Oh43su	71.3	73.3	42.0	60.0	45.3	19.3	27.4	24.0
Hi27su X HSsu	96.0	90.0	49.3	88.0	90.7	9.8	11.2	9.1
Oh43su X B37su	76.0	76.0	26.0	65.3	45.3	23.3	26.5	27.9
HSsu X B37su	95.3	92.0	70.0	90.7	82.7	12.1	13.7	13.9
HSsu X Oh43su	88.7	88.0	67.3	72.0	84.0	17.4	20.2	20.6
Hi27su X Hi38su	93.3	86.0	56.0	97.3	92.0	9.1	13.2	10.7
Hi38su X B37su	96.0	95.3	68.7	89.3	80.0	17.0	13.9	13.7
Hi38su X Oh43su	80.7	93.3	65.3	86.7	73.3	15.9	17.3	15.9
Hi38su X HSsu	98.7	95.3	64.7	96.0	88.0	7.7	10.5	8.1
Hi27bt X B37bt	57.3	52.7	9.3	44.0	25.7	19.4	24.4	19.7
Hi27bt X Oh43bt	59.3	48.0	8.7	50.7	28.0	25.6	36.9	30.7
Oh43bt X B37bt	80.7	41.3	22.7	62.7	42.7	21.8	27.7	27.6
HSbt X Hi27bt	74.0	54.7	40.0	64.0	36.0	18.4	24.5	26.9
HSbt X B37bt	79.3	80.7	42.7	69.3	53.3	17.2	24.6	22.8
HSbt X Oh43bt	93.3	86.7	46.7	82.7	48.0	18.9	17.7	17.3
Hi38bt X Hi27bt	69.3	74.0	44.0	70.7	56.0	10.5	15.7	15.5
Hi38bt X B37bt	86.7	61.3	31.3	69.3	53.3	15.7	21.3	19.4
Hi38bt X Oh43bt	80.7	66.0	28.0	66.7	35.3	17.3	24.4	17.9
Hi38bt X HSbt	83.3	79.3	26.7	94.7	81.3	12.0	16.7	16.1
Hi27sh2 X B37sh2	26.7	24.0	9.3	26.7	13.3	29.5	42.3	39.2
Hi27sh2 X Oh43sh2	51.3	24.0	10.7	34.7	13.3	29.8	41.4	30.1
Hi27sh2 X HSsh2	72.7	44.0	40.0	50.7	24.0	18.2	25.2	24.7
Oh43sh2 X B37sh2	25.3	20.0	6.7	13.3	6.7	32.8	38.8	36.4
HSsh2 X B37sh2	54.7	28.7	13.3	29.3	8.0	29.5	41.3	34.1
HSsh2 X Oh43sh2	78.7	57.3	13.3	53.3	30.7	23.6	30.7	31.0
With Hi38 +	96.9	98.1	70.9	97.2	96.0	12.5	14.1	13.2
With Hi38 *su*	87.9	87.7	55.5	83.2	75.2	14.9	17.3	16.4
With Hi38 *bt*	76.4	64.5	30.0	67.5	46.0	17.7	23.4	21.4
Without Hi38 +	95.9	97.8	69.8	97.1	95.3	13.7	15.6	14.3
Without Hi38 *su*	85.0	84.4	50.0	77.1	69.8	16.5	19.6	19.2
Without Hi38 *bt*	74.0	60.7	28.3	62.2	38.9	20.2	26.0	24.2
Without Hi38 *sh2*	51.6	33.0	15.6	34.7	16.0	27.2	36.6	32.6

moisture content trapped by both paper towel and soil. The average germination rate of wild type hybrids in the cold soil test was also much lower than in the other four tests (Table 4.1). The emergence of field corn usually is not affected too much even in the field with cold soil, therefore the cold soil test used in this study is too severe to predict typical field emergence.

Average germination rates of the four endosperm genotypes for the five germination tests are plotted in Figure 4.1. Averages are given for the six isogenic hybrids (without Hi38*sh2*).

Analyses of variance (Tables 4.2 and 4.3) confirmed the high significance of germination rates among genotypes in different germination tests. This was true both for the 6 pairs of hybrids (without Hi38) and all 36 hybrids (with Hi38). The variances among hybrids within genotypes were also highly significant. Averages, CV's and significant comparisons for each of the germination test are presented in the tables.

Seed conductivity: The data of the standard conductivity test ranged widely from 7.7 to 32.8 milli Siemens/meter, with an overall average of 17 milli S/m (Appendix 2). The data of seed conductivity treated by AA without drying ranged widely from 7.5 to 45.4 milli S/m, with an overall average of 21.3 milli S/m (Appendix 5), and the data of seed conductivity treated by AA after drying

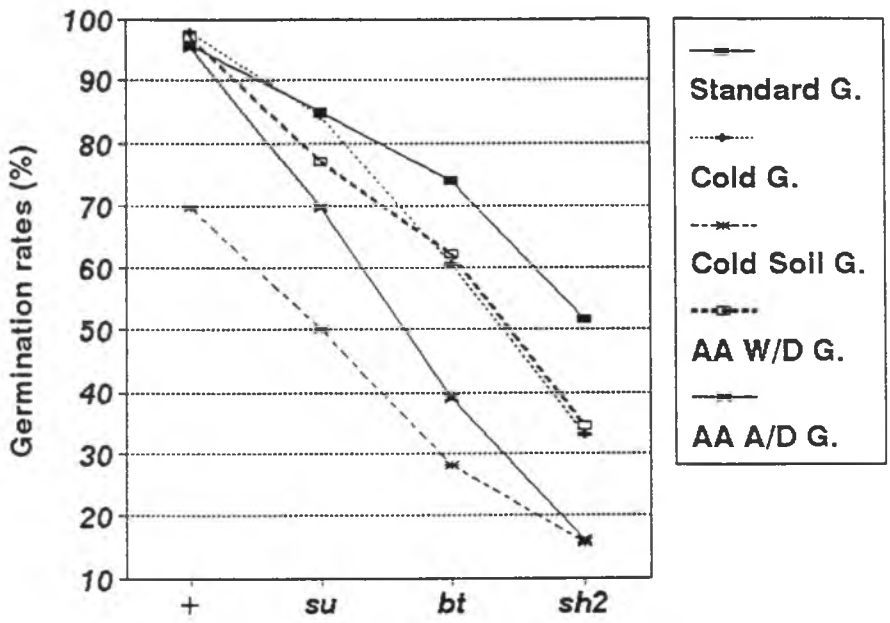

Figure 4.1 The effects of endosperm genes on seed viability estimated by five different germination tests over six hybrids

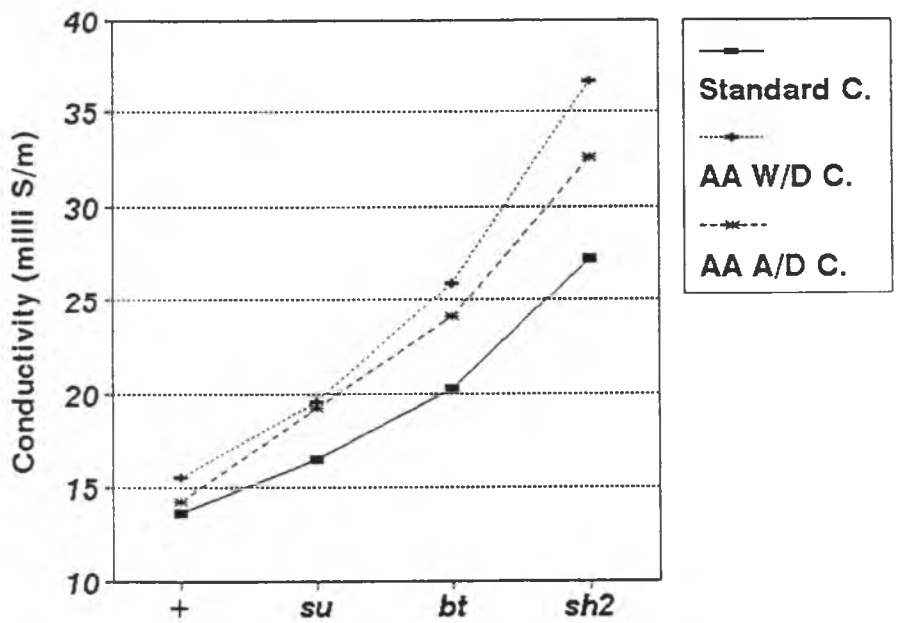

Figure 4.2 The effects of endosperm genes on seed viability estimated by three different conductivity tests over six hybrids

Table 4.2 Analysis of variance of different germination tests

ANOVA: (With Hi38)

		Warm Germ.			Cold Germ.			Cold (soil) Germ.				
	DF	MS	F		MS	F		MS	F		F.05	F.01
Between Genotype	3	1.69	22.8	**	3.41	65.93	**	2.02	24.16	**	2.90	4.46
Betw. Vari. Within Gen	32	0.07	5.554	**	0.05	3.103	**	0.08	3.69	**	1.62	1.98
Between Varieties	35	0.21	15.93		0.34	20.37		0.25	11.01			
Error (Within Variety)	72	0.01			0.02			0.02				
CV =		9.76%			11.5%			20.4%				
Genotype		Average			Average			Average				
+		96.9	a		98.1	a		70.9	a			
su		87.9	b		87.7	b		55.5	b			
bt		76.4	c		64.5	c		30.0	c			
sh2		51.6	d		33.0	d		15.6	d			

ANOVA: (Without Hi38)

		Warm Germ.			Cold Germ.			Cold (soil) Germ.				
	DF	MS	F		MS	F		MS	F		F.05	F.01
Between Genotype	3	1.15	12.69	**	2.53	38.67	**	1.40	13.07	**	3.10	4.94
Betw. Vari. Within Gen	20	0.09	6.341	**	0.07	3.679	**	0.11	4.673	**	1.79	2.28
Between Varieties	23	0.23	16.01		0.39	21.76		0.28	12.03			
Error (Within Variety)	48	0.01			0.02			0.02				
CV =		10.7%			12.8%			22.2%				
Genotype		Average			Average			Average				
+		95.9	a		97.8	a		69.8	a			
su		85.0	ab		84.4	ab		50.0	a			
bt		74.0	b		60.7	b		28.3	b			
sh2		51.6	c		33.0	c		15.6	b			

a. Mean separation by Duncan's test at 5% level.

Table 4.3 Analysis of variance of different AA tests

ANOVA: (With HI38)

		W/out drying AA Warm Germ.			After drying AA Warm Germ.				
	DF	MS	F		MS	F		F.05	F.01
Between Genotype	3	2.86	36.12	**	4.73	63.66	**	2.90	4.46
Betw. Vari. Within Gen	32	0.08	4.535	**	0.07	2.953	**	1.62	1.98
Between Varieties	35	0.32	18.18		0.47	18.81			
Error (Within Variety)	72	0.02			0.03				
CV =		11.9%			16.5%				
Genotype		Average			Average				
+		97.2	a		96.0	a			
su		83.2	b		75.2	b			
bt		67.5	c		46.0	c			
sh2		34.7	d		16.0	d			

ANOVA: (Without HI38)

		W/out drying AA Warm Germ.			After drying AA Warm Germ.				
	DF	MS	F		MS	F		F.05	F.01
Between Genotype	3	2.15	33.42	**	3.50	52.82	**	3.10	4.94
Betw. Vari. Within Gen	20	0.06	3.923	**	0.07	2.085	*	1.79	2.28
Between Varieties	23	0.34	20.51		0.51	16.18			
Error (Within Variety)	48	0.02			0.03				
CV =		12.5%			20.6%				
Genotype		Average			Average				
+		97.1	a		95.3	a			
su		77.1	b		69.8	b			
bt		62.2	c		38.9	c			
sh2		34.7	d		16.0	d			

a. Mean seperation by Duncan's test at 5% level.

ranged similarly from 7.2 to 40.2 milli S/m, with an overall average of 19.6 milli S/m (Appendix 5). The average seed conductivity based on the six isogenic hybrids (without Hi38) and the ten (with Hi38) of +, *su*, *bt*, and *sh2* seeds are summarized in Table 4.1. Data are included for all three conductivity tests.

Analyses of variance (Table 4.4) confirmed the highly significant difference of conductivity among genotypes, in considering both the six and the ten isogenic hybrids. The variances among hybrids within genotypes were also highly significant. Averages, CV's and significant comparisons are presented in this Table.

Comparing the four endosperm hybrids of the same genotypes, in general, there were large differences in germination rate and seed conductivity among the four endosperm types in the five germination tests and the three conductivity tests. The exceptions for these comparisons were between *bt* and *sh2* of the hybrids HS X Hi27, in which differences were among the smallest. Both *bt* and *sh2* counterparts of HS X Hi27 had similar seed weights, similar pericarp thickness, bubble volume (referred to later in Chapter 6), and therefore, similar germination rates and conductivities occurred.

Among the 10 isogenic hybrids, Oh43 X B37 had worse seed quality for unknown reasons. This hybrid did have the thickest pericarps of those tested. The conductivity

Table 4.4 Analysis of variance of different conductivity tests

ANOVA: (With Hi38)

		Standard conductivity			W/out Drying AA Conductivity			After drying AA Conductivity				
	DF	MS	F		MS	F		MS	F		F.05	F.01
Between Genotype	3	884.4	13.50	**	2129	20.80	**	1559	17.74	**	2.9	4.46
Betw. Vari. Within Ge	32	65.52	14.27	**	102.3	12.02	**	87.89	15.31	**	1.62	1.98
Between Varieties	35	135.7	29.56		276.1	32.41		214	37.27			
Error (Within Variety)	72	4.59			8.52			5.74				
CV =		12.6%			13.7%			12.2%				
Genotype		Average			Average			Average				
sh2		27.24	a		36.61	a		32.58	a			
bt		17.68	b		23.39	b		21.38	b			
su		14.87	bc		17.25	c		16.38	bc			
+		12.47	c		14.12	c		13.17	c			

ANOVA: (Without HI38)

		Standard conductivity			W/out Drying AA Conductivity			After drying AA Conductivity				
	DF	MS	F		MS	F		MS	F		F.05	F.01
Between Genotype	3	619.9	10.06	**	1513	13.08	**	1093	11.91	**	3.10	4.94
Betw. Vari. Within Ge	20	61.61	12.53	**	115.7	10.65	**	91.75	13.44	**	1.79	2.28
Between Varieties	23	134.4	27.34		297.9	27.43		222.4	32.57			
Error (Within Variety)	48	4.92			10.86			6.83				
CV =		11.4%			13.5%			11.6%				
Genotype		Average			Average			Average				
sh2		27.24	a		36.61	a		32.58	a			
bt		20.23	b		25.96	ab		24.16	bc			
su		16.50	bc		19.61	b		19.23	cd			
+		13.68	c		15.59	b		14.30	d			

a. Mean seperation by Duncan's test at 5% level.

readings of all the four endosperm genotypes of Oh43 X B37 were among the highest in the three conductivity tests, but Oh43+ X B37+ had very good germination ability even in the cold and AA tests (Table 4.1). This suggested that the conductivity test is more sensitive to detect the unseen defects of seed.

4.2 Accelerated aging (AA)

Germination rates: The germination differences of the two AA tests from the standard germination test for the 36 hybrids are summarized in Table 4.5. These differences are considered as the net responses of the hybrid seeds to AA for germination tests, since there are significant differences of germination rate for different endosperm hybrids even without AA treatments.

In general, hybrids with poor performance at the standard germination test had bigger net response to AA tests. The difference between the standard germination test and the two types of AA germination tests for the + hybrids were either very small or with a negative value, since the wild type hybrids had little responses to AA treatments.

The differences for the *sh2* and *bt* hybrids were the largest. The exceptions were Hi27*sh2* X B37*sh2* and Hi38*bt* X HS*bt*, in which the differences from standard to AA without drying were zero and a negative value (Table 4.5). Hi38*bt* X HS*bt* is a hybrid with good seed quality, and the effect of

Table 4.5 The net responses of germination rates and leachate conductivity to different AA tests

Hybrids	Germination AA W/D Diff.	Germination AA A/D Diff.	Conductivity AA W/D Diff.	Conductivity AA A/D Diff.
Hi27+ X B37+	0.0	1.3	1.4	0.8
Hi27+ X Oh43+	-4.7	-0.7	2.9	0.4
Hi27+ X HS+	-3.3	-4.7	1.1	0.5
Oh43+ X B37+	2.7	6.7	1.5	1.7
HS+ X B37+	-0.7	-0.7	2.7	0.8
HS+ X Oh43+	-1.3	1.3	1.9	-0.5
Hi27+ X Hi38+	5.3	2.7	2.0	1.4
Hi38+ X B37+	2.0	3.3	1.2	-0.4
Hi38+ X Oh43+	-1.3	1.3	1.3	1.2
Hi38+ X HS+	-1.3	-1.3	0.6	1.1
Hi27su X B37su	-4.0	12.0	1.6	2.7
Hi27su X Oh43su	11.3	26.0	8.1	4.8
Hi27su X HSsu	8.0	5.3	1.4	-0.6
Oh43su X B37su	10.7	30.7	3.2	4.6
HSsu X B37su	4.7	12.7	1.5	1.8
HSsu X Oh43su	16.7	4.7	2.8	3.2
Hi27su X Hi38su	-4.0	1.3	4.1	1.6
Hi38su X B37su	6.7	16.0	-3.2	-3.3
Hi38su X Oh43su	-6.0	7.3	1.4	0.1
Hi38su X HSsu	2.7	10.7	2.8	0.3
Hi27bt X B37bt	13.3	31.7	5.1	0.3
Hi27bt X Oh43bt	8.7	31.3	11.3	5.1
Oh43bt X B37bt	18.0	38.0	5.9	5.8
HSbt X Hi27bt	10.0	38.0	6.0	8.5
HSbt X B37bt	10.0	26.0	7.4	5.6
HSbt X Oh43bt	10.7	45.3	-1.2	-1.7
Hi38bt X Hi27bt	-1.3	13.3	5.2	5.0
Hi38bt X B37bt	17.3	33.3	5.6	3.7
Hi38bt X Oh43bt	14.0	45.3	7.0	0.5
Hi38bt X HSbt	*****	2.0	4.8	4.1
Hi27sh2 X B37sh2	0.0	13.3	12.7	9.7
Hi27sh2 X Oh43sh2	16.7	38.0	11.5	0.3
Hi27sh2 X HSsh2	22.0	48.7	7.0	6.5
Oh43sh2 X B37sh2	12.0	18.7	6.0	3.6
HSsh2 X B37sh2	25.3	46.7	11.8	4.6
HSsh2 X Oh43sh2	25.3	48.0	7.2	7.4
+	-0.3	0.9	1.7	0.7
With Hi38 ***su***	4.7	12.7	2.4	1.5
bt	8.9	30.4	5.7	3.7
+	-1.2	0.6	1.9	0.6
Without ***su***	7.9	15.2	3.1	2.7
Hi38 ***bt***	11.8	35.1	5.7	3.9
sh2	16.9	35.6	9.4	5.3

a. The "diff." stands for difference from each datum of AA germination or AA leachate conductivity to its datum of standard treatments.

reduced imbibition damage by the high moisture of AA without drying could be larger than the effect of AA. The difference, therefore, is negligible. The situation of Hi27*sh2* X B37*sh2* is opposite, this hybrid has very poor seed quality, reflected in its poor germination rate and high conductivity under normal conditions. Possibly there could be too little intact membrane left for AA to destroy, thus the net response to AA tends to be relatively small (Table 4.5). Oh43*sh2* X B37*sh2* had a similar situation.

The net response of germination rates of the four endosperm genotypes to both of the AA treatments are shown in Figure 4.3. The wild type corn has the least germination responses to AA treatments, and *sh2* hybrids had the largest responses. Lower germination rates for the four endosperm types occurred with the AA after drying treatments, probably due to more severe imbibition damage (Figure 4.3).

Seed conductivity: The average differences between the normal conductivity test and those for both the AA without drying and AA after drying are also summarized in Table 4.5. These differences are net conductivity to accelerated aging. There were significant differences of conductivity for the four different endosperm genotypes even without AA treatment.

The net conductivity responses of the four endosperm genotypes to both of the AA treatments are shown in Figure 4.4. The wild type corn has the least responses of

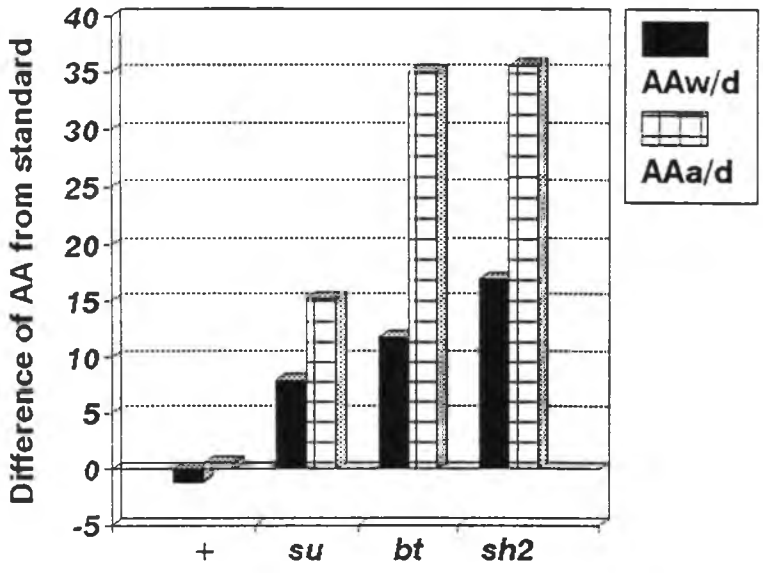

Figure 4.3 Net response of germination to AA for the six isogenic hybrids (without Hi38)

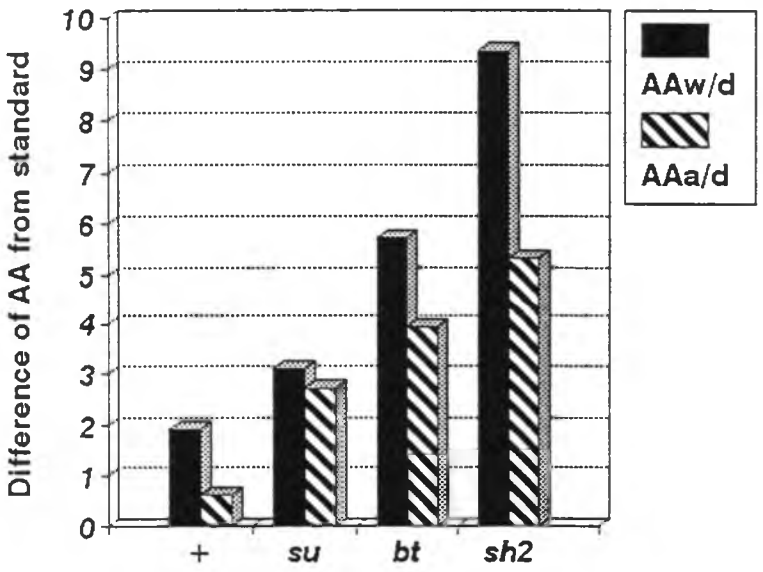

Figure 4.4 Net response of conductivity to AA for the six isogenic hybrids (without Hi38)

conductivity to both of the AA treatments, and the *sh2* hybrids had the largest responses. The conductivity of seed with the AA after drying treatment was unexpectedly lower than the one of seed with the AA without drying (Figure 4.4). This phenomenon suggested that the reduced germination rates related to AA after drying could be caused by some unknown factors other than further loss of seed leachate.

Correlations: The coefficients of determination (r^2) among different germination tests and conductivity tests were all negative and highly significant (Table 4.6). The r^2 values among the five germination tests and among the three conductivity tests were positive and highly significant.

Standard germination and normal conductivity were negatively correlated (r^2 = 66%) even without the influence of AA. Therefore, the values of r^2 between germination and conductivity data from AA seed are confounded by the effects of different endosperm genes.

What are the values of r^2 if the effects of endosperm genotypes were avoided? The values of r^2 between the differences of germination and conductivity from standard to both AA without drying and AA after drying were 22% and 17.3%, respectively. The former was significant at the 1% level and the latter at the 5% level.

Table 4.6 Coefficients of determination among different germination and conductivity tests *(incl. +, su, bt and sh2)*

	A	B	C	D	E	F	G
B	(+) 80.6% **						
C	(+) 63.6% **	(+) 73.8%					
D	(+) 87.4% **	(+) 90.5% **	(+) 70.3%				
E	(+) 76.1% **	(+) 87.7% **	(+) 76.4% *	(+) 91.2%			
F	(-) 66.1% **	(-) 67.4% **	(-) 61.3% *	(-) 74.5% **	(-) 70.7% **		
G	(-) 76.0% **	(-) 81.1% **	(-) 70.8% *	(-) 83.6% **	(-) 79.8% **	(+) 91.5% **	
H	(-) 71.0% **	(-) 75.0% **	(-) 66.5% *	(-) 78.7% **	(-) 75.7% **	(+) 90.2% **	(+) 92.8% **

a. A = Standard germination test, B = Cold germination Test, C = Cold soil germination test, D = AA warm germination (without drying), E = AA warm germination (after drying), F = Standard conductivity test, G = AA conductivity (without drying), H = AA conductivity (after drying).

b. (-) means that the correlation is negative, (+) means that the correlation is positive.

c. *, ** Significant differences at the 5% and 1% levels, respectively.

4.3 Discussion and summary

4.3.1 Viability

Viability and vigor of corn endosperm mutants of near isogenic backgrounds has been studied extensively, although few reports involved the *bt* endosperm gene. The low viability of supersweet (*sh2*) corn is legendary, and the results of this study through different germination and conductivity tests confirms published observations. The *bt* gene, however, is shown to be quite superior in viability to the *sh2* genotype. The + phenotype always performed best, followed in order by *su, bt* and *sh2* corn. Seed viability is influenced by many characters, some of which are discussed in detail in Chapter 6.

4.3.2 Accelerated aging (AA)

The theory that membrane alteration causes deterioration has its basis in the observation that release or leakage of solutes during imbibition can be broadly correlated with aging (Chin and Schoolcraft, 1968; Matthews and Bradnock, 1968; Powell and Matthews, 1978; Parrish and Leopold, 1978). The conductivity test measures electrolytes leached from the seeds upon soaking and estimates the loss of cell membrane integrity.

In this study, the results indicate that seed with poor quality tends to suffer more than those with good quality when passed through AA. The net responses of *sh2* hybrids to

AA for both germination and conductivity tends to be larger than *bt* hybrids, followed in turn by *su* and + hybrids. This tendency is in accord with the performance of the seed with the four endosperm genes in storage at the Waimanalo Experiment Station of the University of Hawaii (Brewbaker, personal communication).

The net responses of germination and conductivity to AA were correlated and significant at the 1% level for seed without drying and at the 5% level for seed after drying. In other words, the electrolyte leakage caused by AA is highly correlated with the deterioration caused by AA. The accelerated aging, therefore, should be useful for predicting seed storability. Seeds with poor germination ability suffer more from AA, and thus, seeds with good germination ability may be expected to have better storability. Germination rate is highly correlated with seed conductivity, and the measurement of seed conductivity is much easier. It is thus possible that seed storability, especially for supersweet corn, could be improved through selecting lines with lower conductivity. Due to the fact that field corn has little net response to AA, keeping near isogenic lines in heterozygous states such as +/*bt* and +/*sh2* would significantly improve their storability.

CHAPTER 5

FOOD QUALITY

5.1 Pericarp thickness

Pericarp thickness was measured for dry seeds harvested at 18 and 36 days after pollination (DAP) for all 36 hybrids and their parental lines, and data are summarized in Appendix 6, 7 and 8. Methods for assessing pericarp thickness (μm) were described in Section 3.2.1. Samples of ten seeds were taken form each hybrid, and their parental lines.

Pericarp thickness averages for the 36 hybrids are summarized in Table 5.1. The data at 18 DAP ranged widely from 46.9 to 143.4 μm, with an overall average of 93.4 μm (Appendix 7), and at 36 DAP from 61.4 to 159.8 μm, with an overall average of 99.8 μm (Appendix 8). For all 36 hybrids at 18 DAP, the average pericarp thicknesses based on the six isogenic hybrids for +, *su*, *bt* and *sh2* seeds were 119.8, 95.5, 85.7 and 87.2 μm (Appendix 7), respectively. For all 36 hybrids at 36 DAP, the average pericarp thicknesses based on the six isogenic hybrids for +, su, *bt* and *sh2* seeds were 90.6, 96.5, 106.0 and 139.7 μm (Appendix 8), respectively. The differences between hybrids at 18 DAP versus those at 36 DAP were highly significant.

Comparing *sh2* with +, *su* and *bt* seeds of the same genotypes for 36 DAP, in general, there were large

Table 5.1 Pericarp thickness (microns) of isogenic hybrids

	18 DAP				36 DAP			
	+	*su*	*bt*	*sh2*	+	*su*	*bt*	*sh2*
Hybrids	Avg	Avg	Avg	Avg	Avg	Avg	Avg	Avg
Hi27 X B37	143.4	109.0	104.1	89.7	113.3	119.4	128.0	159.8
Hi27 X Oh43	122.7	96.1	86.3	84.2	92.0	90.2	109.0	141.7
HS X Oh43	117.1	76.6	74.9	71.0	78.2	97.3	69.3	129.4
Oh43 X B37	118.5	98.5	103.4	93.2	97.8	101.7	121.9	137.3
HS X B37	119.5	108.2	78.5	91.2	85.7	103.6	108.0	159.0
Hi27 X HS	97.9	84.7	67.4	93.8	76.7	67.0	99.7	111.0
Hi27 X Hi38	104.2	88.5	72.8		81.6	72.3	81.9	
Hi38 X HS	85.4	67.3	46.9		80.0	68.0	61.4	
Hi38 X Oh43	99.2	75.3	81.7		83.5	92.9	82.9	
Hi38 X B37	117.4	106.3	87.1		87.7	104.7	98.3	
Avg (With Hi38)	**112.5**	**91.0**	**80.3**		**87.6**	**91.7**	**96.0**	
Avg (W/out Hi38	119.8	**95.5**	**85.7**	**87.2**	**90.6**	**96.5**	**106.0**	139.7

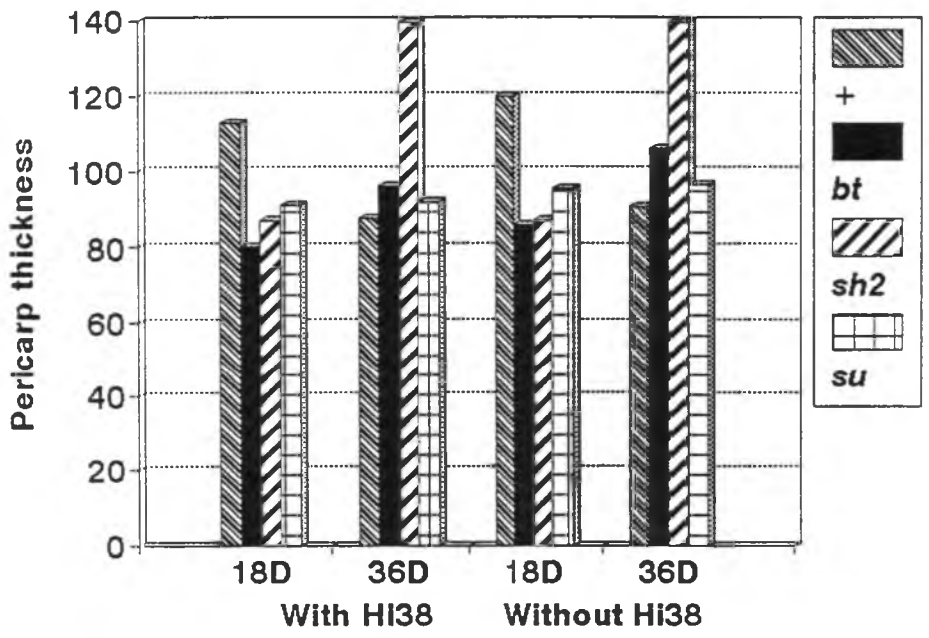

Figure 5.1 Pericarp thickness (microns) of isogenic hybrids at different maturities

differences (> 30 μm). Two exceptions for these comparisons were between *bt* and *sh2* of the hybrids HS X Hi27 and Oh43 X B37, in which differences were small. For HS X Hi27, both *bt* and *sh2* counterparts had similar seed weights (referred to later in Table 6.15), therefore, there was similar kernel inner pressure on the pericarp, which resulted in similar pericarp thickness. For Oh43 X B37, there was a large difference in seed weight between their *bt* and *sh2* counterparts (Table 6.15), but the difference of pericarp thickness (15.4 μm) was relatively small. The reason for this is not quite clear.

The significant comparison in pericarp thickness of the four genotypes at different maturities is evident in Figure 5.1. Averages are given for all 10 + hybrids, 10 *su* hybrids, 10 *bt* hybrids and for the 6 *sh2* hybrids without Hi38*sh2* at both 18 DAP and 36 DAP.

Analyses of variance (Table 5.2) confirmed the significance of differences between genotypes, in considering both the 6 pairs of hybrids and all 16 hybrids. In comparing the averages of +, *su*, *bt* and *sh2* hybrids, there were no significant differences between *bt*, *sh2* and *su* for hybrids at 18 DAP, and no significant difference between +, *bt* and *su* for hybrids at 36 DAP. The variances among hybrids within genotypes were also highly significant. Error variances were not very high, resulting in CV's of 11.74% and 13.33% for 18 DAP and 36 DAP (without Hi38),

Table 5.2 Analysis of variance of pericarp thickness (microns) for different endosperm genotypes

ANOV (Without Hi38):

Source	DF	18 DAP MS	18 DAP F		36 DAP MS	36 DAP F		F.05	F.01
Between Genotypes	3	14958	8.8	**	28835	9.2	**	3.10	4.94
Betw. Variety within Geno.	20	1703	13.1	**	3150	15.1	**	1.62	1.97
Between Varieties	23	3432	26.4		6500.2	31.2			
Error (Within Varieties)	216	129.78			208.08				
	LSD.05 =		15.7			21.4			
	LSD.01 =		21.4			29.2			
	CV =		11.74%			13.33%			

Genotype	Average		Genotype	Average	
+	119.8	A	*sh2*	139.7	A
su	95.5	B	*bt*	106.0	B
sh2	87.2	B	*su*	96.5	B
bt	85.7	B	+	90.6	B

ANOV (With Hi38):

Source	DF	18 DAP MS	18 DAP F		36 DAP MS	36 DAP F		F.05	F.01
Between Genotypes	3	18882	8.3	**	39406	12.8	**	2.90	4.46
Betw. Variety within Geno.	32	2281	19.0	**	3089.5	17.7	**	1.49	1.74
Between Varieties	35	3703.9	30.8		6202.3	35.4			
Error (Within Varieties)	324	120.25			175.01				
	LSD.05 =		14.5			16.9			
	LSD.01 =		19.5			22.7			
	CV =		11.74%			13.26%			

Genotype	Average		Genotype	Average	
+	112.5	A	*sh2*	139.7	A
su	91.0	B	*bt*	96.0	B
sh2	87.2	B	*su*	91.7	B
bt	80.3	B	+	87.6	B

a. Mean seperation by Duncan's test at 1% level.

respectively, and 11.74% and 13.26% for 18 DAP and 36 DAP (with Hi38), respectively. Averages and significant comparisons are presented in Table 5.2.

The pericarp thicknesses of parental lines (Appendix 6) also showed the influences of endosperm genes on pericarps. The thickest pericarps always occurred in lines with the *sh2* gene regardless of their genotypes, and was followed by lines with *bt* endosperm genes. The exception was HS*bt*, that had the thinnest pericarp in comparison to its +, *su* and *sh2* counterparts. The HS*bt* used in this study is actually a variety named "Hawaiian Supersweet #9" which has undergone several cycles of mass selections to improve its tenderness (Ito and Brewbaker, 1981), probably accounting for its very thin pericarp.

5.2 Sensory evaluation

Many characters that relate to the eating quality of supersweet corn (*bt* and *sh2*) could be affected by the endosperm genotypes. Four of the characters were considered in this study. These were tenderness, flavor, sweetness, and crispness. The effects of endosperm genes on these characters were based on 6 *sh2* hybrids, 10 *bt* hybrids and 10 *su* hybrids. The comparison among different endosperm genes was conducted with equal (without Hi38) and unequal (with Hi38*bt*, *sh2* and *su*) subclass numbers of hybrids. For each of the two cases, the sensory evaluations were conducted on

both fresh and microwave steamed ears that were harvested 18 days after pollination. The evaluation of the four characters was based on a 1 to 9 hedonic scale, with 1 representing the best quality and 9 representing the worst.

5.2.1 Tenderness

Tenderness was evaluated for both fresh and cooked ears of 26 hybrids and is summarized in Appendix 14. Methods for assessing tenderness were described in Section 3.2.2.

Tenderness averages for the 26 hybrids are summarized in Table 5.3. The data ranged widely from 2.61 to 7.89, with an overall average of 6.32 for fresh ears, and from 2.11 to 7.0, with an overall average of 5.27 for cooked ears (Appendix 14). For fresh ears of *bt, sh2,* and *su,* the average tenderness based on the six isogenic hybrids were 6.67, 6.81, and 6.78, respectively; for cooked ears were 5.63, 5.76, and 5.54, respectively. These differences for both fresh and cooked ears were not significant. The hybrid HS*bt* X Hi27*bt* had unusually good tenderness compared to *sh2* and *su* NILs, possibly due to its thin pericarp and lower starch content.

The comparison in tenderness of the three genotypes is shown in Figure 5.2. Averages are given for all 10 *bt* and 10 *su* hybrids and for the 6 hybrids (without Hi38) with counterpart *sh2* types for both fresh and cooked ears.

Analyses of variance (Table 5.4) confirmed that there

Table 5.3 Hedonic scores of tenderness for sweet and supersweet corn on a 1 to 9 scale

	Fresh			Cooked		
	su	*bt*	*sh2*	*su*	*bt*	*sh2*
Hybrids	Avg	Avg	Avg	Avg	Avg	Avg
Hi27 X B37	7.78	7.72	7.61	6.00	6.72	5.94
Hi27 X Oh43	6.22	6.89	7.67	4.67	5.67	5.94
Oh43 X B37	7.89	8.06	7.50	6.89	7.00	5.56
HS X B37	7.44	6.89	5.17	6.67	6.28	5.56
HS X Oh43	5.89	6.67	6.56	5.00	5.89	5.06
HS X Hi27	4.78	4.61	6.17	4.56	3.00	5.17
Hi38 X Hi27	5.33	5.56		3.44	5.56	
Hi38 X HS	3.00	2.61		2.78	2.11	
Hi38 X B37	6.89	7.67		5.89	6.28	
Hi38 X Oh43	5.00	6.72		4.11	5.28	
Avg (With Hi38)	**6.02**	**6.34**		**5.00**	**5.38**	
Avg (Without Hi38)	**6.67**	**6.81**	**6.78**	**5.63**	**5.76**	**5.54**

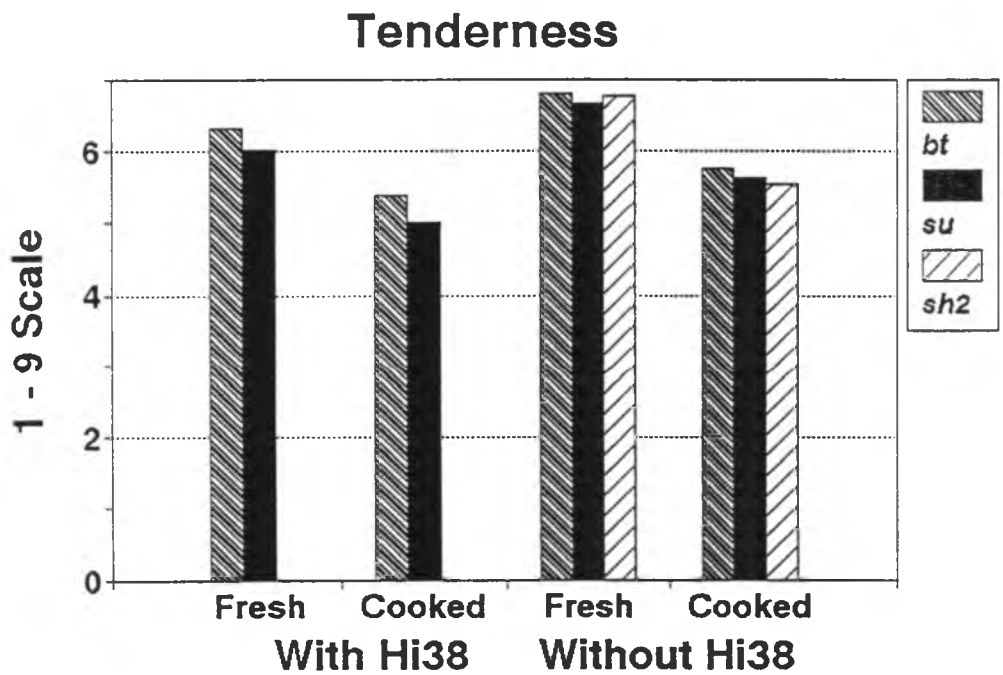

Figure 5.2 The difference of tenderness among endosperms of su, bt and sh2 on a 1 to 9 scale

Table 5.4 Analysis of variance of tenderness (1 to 9 scale) for different endosperm genotypes

ANOV: (Without Hi38)

Source	DF	Fresh MS	Fresh F		Cooked MS	Cooked F		F.05	F.01
Between Genotypes	2	0.0972	0.02		0.2243	0.07		3.68	6.36
Betw. Variety within Geno.	15	3.996	12.138	**	3.2704	18.699	**	1.98	2.62
Between Varieties	17	3.5373	10.745		2.912	16.65			
Error (Within Varieties)	36	0.3292			0.1749				
	LSD.05 =		1.42			1.29			
	LSD.01 =		1.96			1.78			
	CV =		8.50%			7.41%			

Genotype	Average	Genotype	Average
bt	6.81	*bt*	5.76
sh2	6.78	*su*	5.63
su	6.67	*sh2*	5.54

ANOV: (With Hi38)

Source	DF	Fresh MS	Fresh F		Cooked MS	Cooked F		F.05	F.01
Between Genotypes	2	3.2214	0.4786		1.9095	0.3654		3.42	5.66
Betw. Variety within Geno.	23	6.7308	26.177	**	5.2258	20.903	**	1.74	2.18
Between Varieties	25	6.45	25.085		4.9605	19.842			
Error (Within Varieties)	52	0.2571			0.25				
	LSD.05 =		1.49			1.31			
	LSD.01 =		2.02			1.78			
	CV =		8.03%			9.49%			

Genotype	Average	Genotype	Average
sh2	6.78	*sh2*	5.54
bt	6.34	*bt*	5.38
su	6.02	*su*	5.00

were no significant differences between genotypes, in considering both the 6 pairs of hybrids and all 26 hybrids. The variances among hybrids within genotypes were highly significant. Error variances were not very high, resulting in CV's of 8.50% and 7.41% for fresh and cooked ears (without Hi38), respectively, and 8.03% and 9.49% for fresh and cooked ears (with Hi38), respectively. Averages are presented in Table 5.3.

5.2.2 Sweetness

Sweetness was evaluated for both fresh and cooked ears of all 26 hybrids (Appendix 15). Methods for assessing sweetness were described in Section 3.2.2.

Sweetness averages for the 26 hybrids are summarized in Table 5.5. The data ranged widely from 1.67 to 7.56, with an overall average of 5.34 for fresh ears, and from 1.78 to 7.44, with an overall average of 5.61 for cooked ears (Appendix 15). For fresh ears of *su, bt* and *sh2*, the average sweetness based on an equal number of hybrids was 7.19, 5.20, and 3.69, respectively; for cooked ears, the average sweetness was 7.11 , 5.75, and 3.80, respectively. These differences for both fresh and cooked ears were highly significant.

In comparing the six isogenic hybrids of *su, bt*, and *sh2* seeds, *sh2* hybrids were sweeter than *bt* hybrids for both fresh and cooked ears, and *su* hybrids had the worst

Table 5.5 Hedonic scores of sweetness for sweet and supersweet corn on a 1 to 9 scale

	Fresh			Cooked		
	su	*bt*	*sh2*	*su*	*bt*	*sh2*
Hybrids	Avg	Avg	Avg	Avg	Avg	Avg
Hi27 X B37	7.56	5.22	4.17	7.44	6.06	3.89
Hi27 X Oh43	6.56	5.39	4.00	6.44	5.72	4.28
Oh43 X B37	6.56	5.67	3.61	7.33	6.61	3.67
HS X B37	7.56	5.67	2.72	6.78	6.83	2.78
HS X Oh43	7.11	7.06	3.50	7.00	6.83	3.89
HS X Hi27	7.78	2.22	4.11	7.67	2.44	4.28
Hi38 X Hi27	6.78	2.94		7.44	4.56	
Hi38 X HS	5.44	1.67		7.22	1.78	
Hi38 X B37	7.00	5.94		7.22	6.11	
Hi38 X Oh43	6.33	6.17		5.89	5.61	
Avg (With Hi38)	**6.87**	**4.79**		**7.04**	**5.26**	
Avg (Without Hi38)	**7.19**	**5.20**	**3.69**	**7.11**	**5.75**	**3.80**

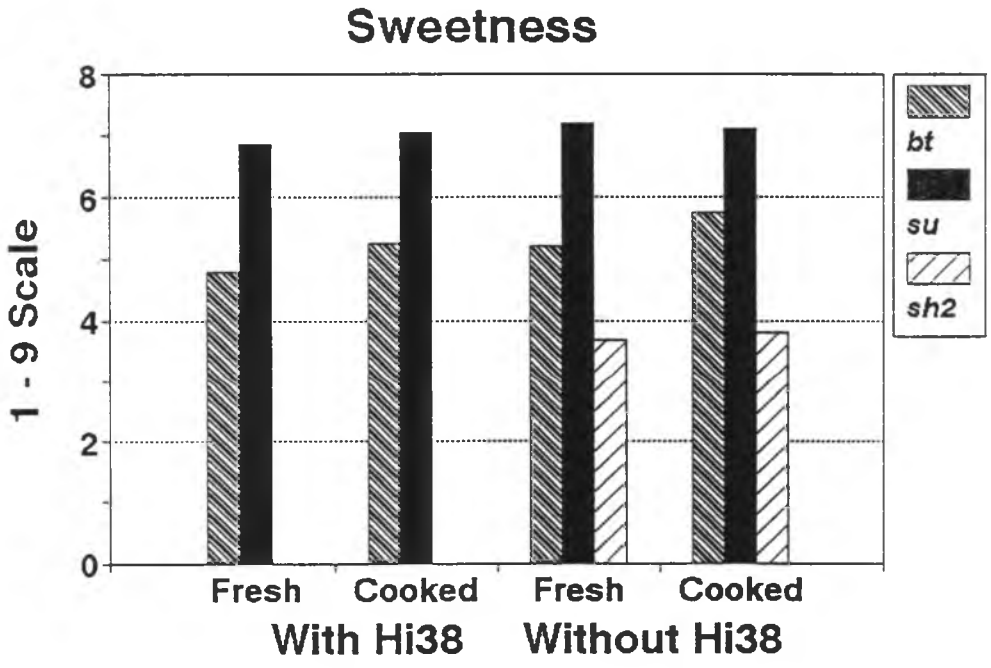

Figure 5.3 The difference of sweetness among endosperms of su, bt and sh2 on a 1 to 9 scale

sweetness. One exception to this comparison was the hybrid HS X Hi27, in which the *bt* counterpart was much sweeter than its *sh2* counterpart and other *bt* hybrids. The extensive genotypic variability for sweetness among the *bt* hybrids indicated that allelic variation at loci other than *bt* is involved.

The significant comparison in sweetness of the three genotypes is evident in Figure 5.3. Averages are given for all 10 *bt* and 10 *su* hybrids and for the 6 hybrids with counterpart *sh2* types for both fresh and cooked ears.

Analyses of variance (Table 5.6) confirmed the significance of differences between genotypes, in considering both the 6 pairs of hybrids and all 16 hybrids. The variances among hybrids within genotypes were also highly significant. Error variances were not very high, resulting in CV's of 7.77% and 7.78% for fresh and cooked ears (without Hi38), respectively, and 7.79% and 7.08% for fresh and cooked ears (with Hi38), respectively. Averages and significant comparisons are also presented in Table 5.6.

5.2.3 Flavor

Flavor was evaluated for both fresh and cooked ears of all 26 hybrids and is summarized in Appendix 16. Methods for assessing flavor were described in Section 3.2.2.

Flavor averages for the 26 hybrids are summarized in

Table 5.6 Analysis of variance of sweetness (1 to 9 scale) for different endosperm genotypes

ANOV: (Without Hi38)

Source	DF	Fresh MS	Fresh F		Cooked MS	Cooked F		F.05	F.01
Between Genotypes	2	55.447	17.67	**	49.973	14.98	**	3.68	6.36
Betw. Variety within Geno.	15	3.137	18.096	**	3.3363	17.867	**	1.98	2.62
Between Varieties	17	9.2911	53.596		8.823	47.25			
Error (Within Varieties)	36	0.1734			0.1867				
LSD.05 =			1.26			1.30			
LSD.01 =			1.74			1.79			
CV =			7.77%			7.78%			

Genotype	Average		Genotype	Average	
su	7.19	a	*su*	7.11	a
bt	5.20	b	*bt*	5.75	b
sh2	3.69	b	*sh2*	3.80	b

ANOV: (With Hi38)

Source	DF	Fresh MS	Fresh F		Cooked MS	Cooked F		F.05	F.01
Between Genotypes	2	64.07	13.628	**	62.354	14.409	**	3.42	5.66
Betw. Variety within Geno.	23	4.7012	27.219	**	4.3274	27.43	**	1.74	2.18
Between Varieties	25	9.4507	54.717		8.9696	56.855			
Error (Within Varieties)	52	0.1727			0.1578				
LSD.05 =			1.24			1.19			
LSD.01 =			1.69			1.62			
CV =			7.79%			7.08%			

Genotype	Average		Genotype	Average	
su	6.87	a	*su*	7.04	a
bt	4.79	b	*bt*	5.26	b
sh2	3.69	b	*sh2*	3.80	b

a. Mean seperation by Duncan's test at 5% level.

Table 5.7. The data ranged widely from 2.44 to 7.11, with a somewhat better average of 5.41 for fresh ears, and from 2.17 to 7.11 with an overall average of 5.08 for cooked ears (Appendix 16). For fresh ears of *su, bt,* and *sh2*, the average flavor based on the six isogenic hybrids was 6.64, 5.38, and 5.04, respectively. For cooked ears, the average flavor was 6.04, 5.29, and 4.25, respectively. These differences for both fresh and cooked ears were significant. In comparing *su, bt,* and *sh2* seeds of the same genotype, there was no significant difference between *bt* and *sh2* for both fresh and cooked ears.

The significant difference in flavor of the three genotypes is evident in Figure 5.4. Averages are given for all 10 *bt* hybrids, all 10 *su* hybrids, and for the 6 hybrids with counterpart *sh2* types for both fresh and cooked ears. In every case the sugary lines were given the worst flavor ratings.

Analyses of variance (Table 5.8) confirmed the significant differences between genotypes, in considering both the 6 pairs of hybrids and all 16 hybrids. The variances among hybrids within genotypes were highly significant. Error variances were not very high, resulting in CV's of 9.03% and 7.82% for fresh and cooked ears (without Hi38), respectively, and 8.74% and 8.66% for fresh and cooked ears (with Hi38), respectively. Averages and significant comparisons are presented in Table 5.8.

Table 5.7 Hedonic scores of flavor for sweet and supersweet corn on a 1 to 9 scale

	Fresh			Cooked		
	su	*bt*	*sh2*	*su*	*bt*	*sh2*
Hybrids	Avg	Avg	Avg	Avg	Avg	Avg
Hi27 X B37	6.89	5.61	5.39	6.22	5.39	4.17
Hi27 X Oh43	5.67	5.89	5.22	5.33	5.44	4.61
Oh43 X B37	6.33	6.61	4.89	6.33	6.17	4.33
HS X B37	7.11	5.94	4.50	5.67	5.56	3.94
HS X Oh43	6.33	5.22	5.28	5.56	6.61	4.11
HS X Hi27	6.44	3.00	4.94	7.11	2.56	4.33
Hi38 X Hi27	5.67	3.83		6.11	4.50	
Hi38 X HS	3.89	2.44		4.44	2.17	
Hi38 X B37	6.33	5.94		6.44	5.78	
Hi38 X Oh43	4.78	6.39		4.00	5.28	
Avg (With Hi38)	**5.94**	**5.09**		**5.72**	**4.94**	
Avg (Without Hi38)	**6.46**	**5.38**	**5.04**	**6.04**	**5.29**	**4.25**

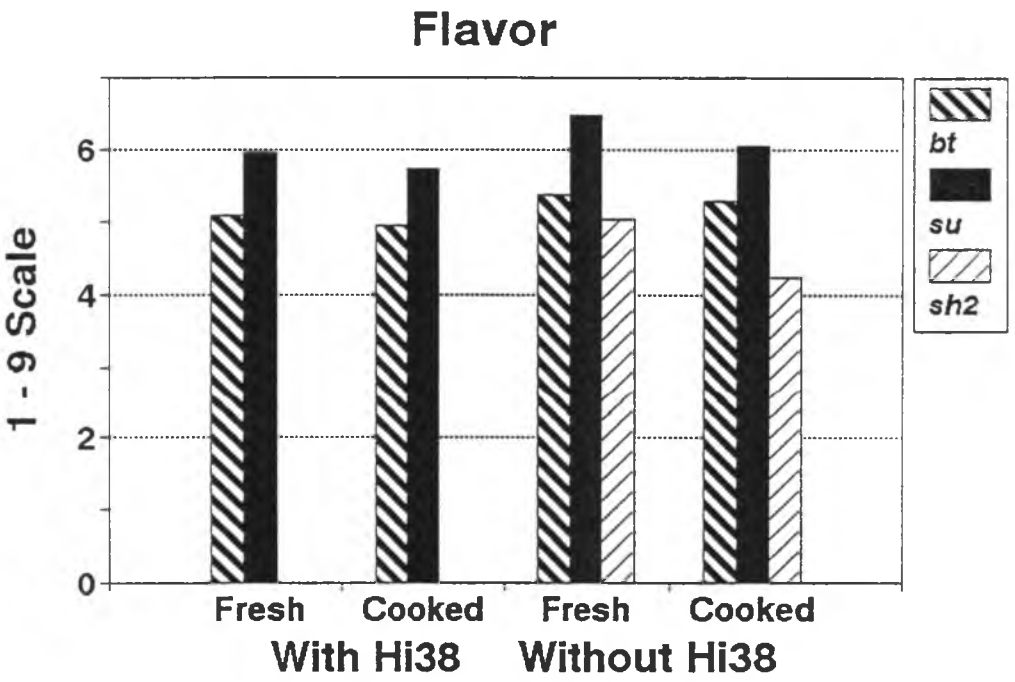

Figure 5.4 The difference of flavor among endosperms of su, bt and sh2 on a 1 to 9 scale

Table 5.8 Analysis of variance of flavor (1 to 9 scale) for different endosperm genotypes

ANOV: (Without Hi38)

Source	DF	Fresh MS	Fresh F		Cooked MS	Cooked F		F.05	F.01
Between Genotypes	2	9.97	5.17	*	14.494	5.80	*	3.68	6.36
Betw. Variety within Geno.	15	1.93	7.47	**	2.50	15.19	**	1.98	2.62
Between Varieties	17	2.87	11.13		3.91	23.76			
Error (Within Varieties)	36	0.26			0.16				
LSD.05 =		1.00				1.12			
LSD.01 =		1.36				1.55			
CV =		9.03%				7.82%			

Genotype	Average		Genotype	Average	
su	6.46	a	*su*	6.04	a
bt	5.38	b	*bt*	5.29	ab
sh2	5.04	b	*sh2*	4.25	b

ANOV: (With Hi38)

Source	DF	Fresh MS	Fresh F		Cooked MS	Cooked F		F.05	F.01
Between Genotypes	2	7.08	1.90		12.662	3.49	*	3.42	5.66
Betw. Variety within Geno.	23	3.73	16.69	**	3.62	18.71	**	1.74	2.18
Between Varieties	25	3.99	17.89		4.35	22.44			
Error (Within Varieties)	52	0.22			0.19				
LSD.05 =		1.11				1.09			
LSD.01 =		1.50				1.48			
CV =		8.74%				8.66%			

Genotype	Average		Genotype	Average	
su	5.94		*su*	5.72	a
bt	5.09		*bt*	4.94	ab
sh2	5.04		*sh2*	4.25	b

a. Mean seperation by Duncan's test at 5% level.

5.2.4 Crispness

Crispness was evaluated for both fresh and cooked ears of all 26 hybrids and is summarized in Appendix 17. Methods for assessing crispness were described in Section 3.2.2.

Crispness averages for the 26 hybrids are summarized in Table 5.9. The data ranged widely from 3.72 to 7.33, with an overall average of 5.37 for fresh ears, and from 4.33 to 7.78, with an overall average of 6.33 for cooked ears (Appendix 17). For fresh ears the average crispness based on the six isogenic hybrids for *su, bt,* and *sh2* seeds were 6.72, 4.59, and 4.39, respectively; for cooked ears were 7.24, 6.07, and 5.47, respectively, and these differences for both fresh and cooked ears were highly significant. In comparing *bt, sh2,* and *su* seeds of the same genotype, there were no significant differences between *bt* and *sh2* hybrids for both fresh and cooked ears, while *su* hybrids had the worst crispness.

The significant difference in crispness of the three genotypes is evident in Figure 5.5. Averages are given for all 10 *bt* and 10 *su* hybrids and for the 6 hybrids with counterpart *sh2* types for both fresh and cooked ears.

Analyses of variance (Table 5.10) confirmed that there were highly significant differences between genotypes, in considering both the 6 pairs of hybrids and all 16 hybrids. The variances among hybrids within genotypes were also highly significant. Error variances were very low,

Table 5.9 Hedonic scores of crispness for sweet and supersweet corn on a 1 to 9 scale

	Fresh			Cooked		
	su	*bt*	*sh2*	*su*	*bt*	*sh2*
Hybrids	Avg	Avg	Avg	Avg	Avg	Avg
Hi27 X B37	7.33	4.72	4.83	7.56	6.56	5.72
Hi27 X Oh43	6.33	4.78	4.67	7.00	6.17	5.67
Oh43 X B37	6.33	4.89	3.94	6.78	6.17	5.22
HS X B37	7.22	4.61	4.39	7.56	6.67	5.00
HS X Oh43	6.44	4.39	4.06	7.11	6.56	5.22
HS X Hi27	6.67	4.17	4.50	7.44	4.33	6.00
Hi38 X Hi27	6.78	4.83		7.33	5.67	
Hi38 X HS	7.22	3.72		6.89	4.89	
Hi38 X B37	7.00	4.56		7.44	6.17	
Hi38 X Oh43	6.56	4.67		7.78	5.78	
Avg (With Hi38)	**6.79**	**4.53**		**7.29**	**5.89**	
Avg (Without Hi38)	**6.72**	**4.59**	**4.40**	**7.24**	**6.07**	**5.47**

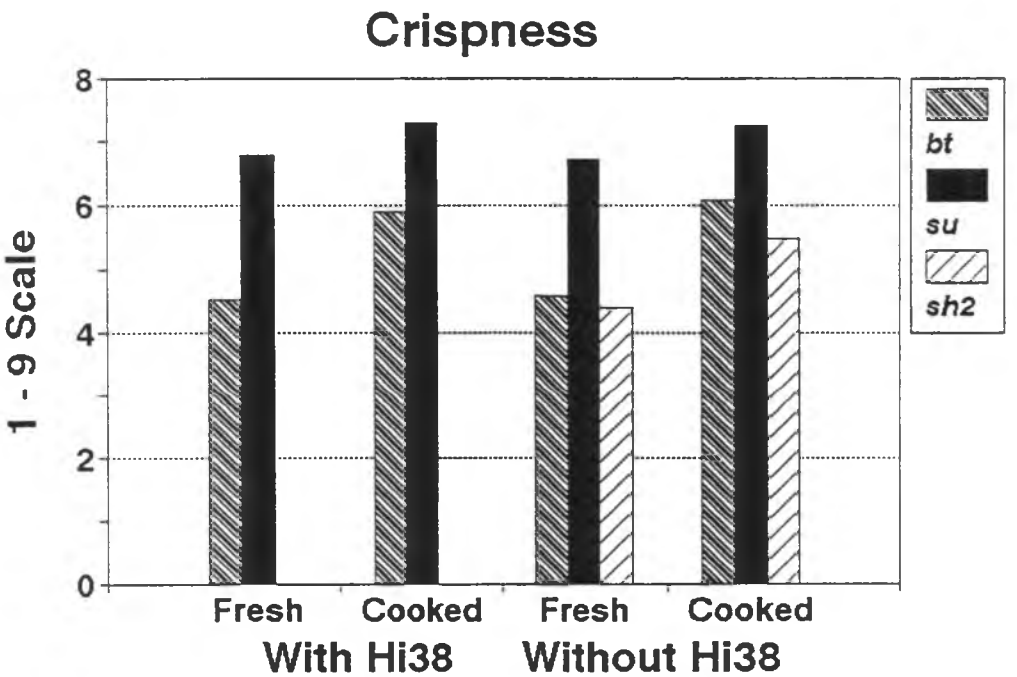

Figure 5.5 The difference of crispness among endosperms of su, bt and sh2 on a 1 to 9 scale

Table 5.10 Analysis of variance of crispness (1 to 9 scale) for different endosperm genotypes

ANOV: (Without Hi38)

Source	DF	Fresh MS	Fresh F		Cooked MS	Cooked F		F.05	F.01
Between Genotypes	2	29.923	76.20	**	14.553	14.22	**	3.68	6.36
Betw. Variety within Geno.	15	0.3927	6.4695	**	1.0236	8.6891	**	1.98	2.62
Between Varieties	17	3.8669	63.705		2.6153	22.201			
Error (Within Varieties)	36	0.0607			0.1178				
LSD.05 =		0.45			0.72				
LSD.01 =		0.62			0.99				
CV =		4.70%			5.48%				

Genotype	Average		Genotype	Average	
su	6.72	a	*su*	7.24	a
bt	4.59	b	*bt*	6.07	b
sh2	4.40	b	*sh2*	5.47	b

ANOVA: (With Hi38)

Source	DF	Fresh MS	Fresh F		Cooked MS	Cooked F		F.05	F.01
Between Genotypes	2	49.199	123.09	**	23.259	25.839	**	3.42	5.66
Betw. Variety within Geno.	23	0.3997	4.9881	**	0.9002	8.2603	**	1.74	2.18
Between Varieties	25	4.3037	53.71		2.6889	24.675			
Error (Within Varieties)	52	0.0801			0.109				
LSD.05 =		0.36			0.54				
LSD.01 =		0.49			0.74				
CV =		5.27%			5.21%				

Genotype	Average		Genotype	Average	
su	6.79	a	*su*	7.29	a
bt	4.53	b	*bt*	5.89	b
sh2	4.40	b	*sh2*	5.47	b

a. Mean seperation by Duncan's test at 5% level.

resulting in CV's of 4.70% and 5.48% for fresh and cooked ears (without Hi38), respectively, and 5.27% and 5.21% for fresh and cooked ears (with Hi38), respectively. Averages and significant comparisons are presented in Table 5.10.

5.2.5 The differences between fresh and cooked ears

The differences between fresh and steam-cooked ears were compared through a split-split plot design based on the six isogenic hybrids for the three endosperm genotypes. Fresh and cooked ears were considered as the mainplots, with the endosperm genotypes as the subplots and the hybrids as the sub-subplots.

The differences between fresh and steam-cooked ears for flavor were significant. There was no significant difference of flavor between fresh and steam-cooked ears for the *bt* hybrids. The differences of flavor between the fresh and steam-cooked ears for the *sh2* and *su* hybrids were highly significant and significant, respectively (Table 5.11).

The difference between fresh and steam-cooked ears for sweetness was not significant. There was no significant difference of sweetness between the fresh and steam-cooked ears for both of the *sh2* and *su* hybrids. The difference of sweetness between the fresh and steam-cooked ears for the *bt* hybrids was significant.

The difference between fresh and steam-cooked ears for tenderness was significant. After steam-cook, the

Table 5.11 Analysis of variance (Split-split-plot design) of the four eating qualities.

ANOV

Source	df	Flavor MS	Flavor F		Sweetness MS	Sweetness F		Tenderness MS	Tenderness F		Crispness MS	Crispness F		F.05	F.01
Treatment (T)	1	5.11	49.83	*	1.02	4.00		33.15	38.97	*	28.35	208.4	**	18.51	98.49
Error(a)	2	0.10			0.26			0.85			0.14				
Endosperm (E	2	23.38	122.8	**	104.5	320.8	**	0.20	1.59		42.37	532.3	**	3.49	5.95
T*E	2	1.09	5.70	*	0.91	2.80	**	0.12	0.93		2.10	26.42	**	3.49	5.95
Error(b)	8	0.19			0.33			0.13			0.08				
Varieties (V)	5	2.35	13.42	**	2.83	22.86	**	13.82	54.37	**	0.97	11.81	**	2.33	3.25
T*V	5	0.33	1.90		0.18	1.49		0.88	3.45	**	0.13	1.57		2.33	3.25
E*V	10	4.60	26.31	**	7.87	63.50	**	3.11	12.23	**	1.13	13.80	**	1.80	2.28
T*E*V	10	0.70	4.00	**	0.34	2.72	**	0.44	1.74		0.45	5.50	**	1.99	2.63
Error(c)	60	0.17			0.12			0.25			0.08				
Total	107														
cv (a) =		5.92%			9.26%			14.9%			6.42%				
cv (b) =		8.07%			10.5%			5.76%			4.91%				
cv (c) =		7.73%			6.45%			8.14%			4.97%				
Endosperm		Average			Average			Average			Average				
Fresh bt		5.38			5.20			6.81			4.59				
Fresh sh2		5.04			3.69			6.78			4.40				
Fresh su		6.46			7.19			6.67			6.72				
Cooked bt		5.29			5.75			5.76			6.07				
Cooked sh2		4.25			3.80			5.54			5.47				
Cooked su		6.04			7.11			5.63			7.24				
Difference															
F. bt vs. C. bt		ns			*			**			**				
F. sh2 vs. C. sh2		**			ns			**			**				
F. su vs. F. su		*			ns			**			**				

a. Mainplot = Treatments (Fresh vs. Cooked).
Subplot = Endosperm types.
Sub-subplot = Varieties.

b. df = degree of freedom.

c. *, ** Significant differences at the 5% and 1% levels, respectively.

tenderness improved for all of the three endosperm genotypes. There was highly significant differences between fresh and steam-cooked ears for the *su, bt* and *sh2* hybrids.

The difference between fresh and steam-cooked ears for crispness was highly significant. The crispness of steam-cooked ears decreased drastically, especially for the *sh2* and *bt* hybrids. There were highly significant differences in crispness between fresh and steam-cooked ears for the *bt, sh2* and *su* hybrids (Table 5.11).

5.3 Correlations

5.3.1 Correlations for Pericarp thickness and seed weight

Average pericarp thickness at 36 DAP were greater than that at 18 DAP for the hybrids with endosperm genes *su, bt* and *sh2*. There was an adverse condition for the hybrids with wild type endosperm (Table 5.1). It was found that the correlation of seed weights with the differences between pericarp thickness of 36 DAP and that of 18 DAP was negative and highly significant (r^2= 74%) (Figure 5.6). Since the pericarp thickness at 36 DAP was thinner than that at 18 DAP for the hybrids with wild type endosperm, the difference in pericarp thickness for the wild type hybrids had negative values. Consequently, there were highly significant correlations between seed weight and differences for pericarp thickness of the germinal side at 36 DAP minus that at 18 DAP (r^2 = 70.4%) and for pericarp thickness of the

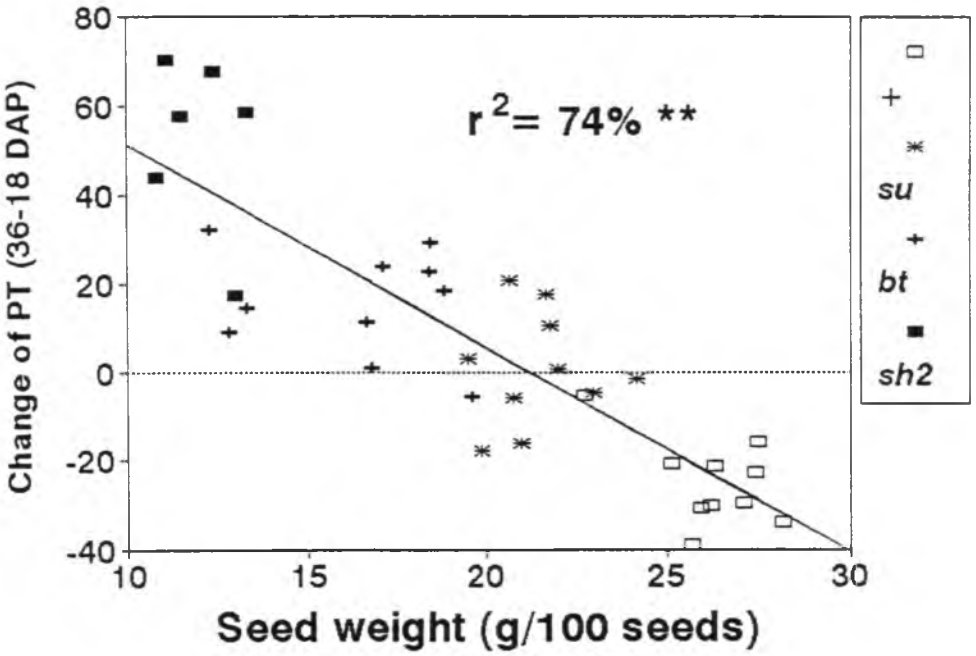

Figure 5.6 Correlation between change of PT (36-18 DAP) and seed weight

abgerminal side at 36 DAP minus that at 18 DAP (r^2 = 70.0%) (Table 5.12). These phenomena suggested that the changes of pericarp thickness were strongly influenced by seed weight, which became more and more important to determine the seed inner pressure since seed moisture was gradually decreased during the stage of 18 to 36 DAP.

5.3.2 Correlations for sensory evaluation

The relationships among the four different eating qualities and pericarp thickness at 18 days after pollination (DAP) were estimated by the coefficients of determination (r^2) and are presented in Table 5.13. Sweetness was found to be the principal component of flavor (Boyer and Shannon, 1983). Correlation coefficients between sugar content and flavor measured by taste panel scores were higher when the corn was relatively lower in sugar (Winter, 1955). The sweetness of the *bt* and *sh2* hybrids used in this study were generally ranked at a lower level, except for the three hybrids with Hawaii-bred parental lines. The coefficient of determination (r^2) of flavor with sweetness was 77% and highly significant. The correlations between flavor and the other two eating qualities as well as pericarp thickness (18 DAP) were all highly significant despite the difference in r^2 values (Table 5.13). This suggests that flavor is a character strongly influenced by sweetness, tenderness, crispness, and pericarp thickness.

Table 5.12 Coefficients of determination between the differences of PT (36 DAP - 18 DAP) and seed weight

Difference of germinal side PT (36-18) vs. seed weight	(-)	70.4% **
Difference of abgerminal side PT (36-18) vs. seed weight	(-)	70.0% **
Difference of averge PT (36-18) vs. seed weight	(-)	74.0% **

a. *, ** significant difference at 5% and 1% levels, respectively.

Table 5.13 Coefficients of determination among the four characters of eating quality

	Flavor	Sweetness	Tenderness	Crispness
Sweetness	76.6% **			
Tenderness	43.6% **	10.6%		
Crispness	38.7% **	73.9% **	0.2%	
PT of 18 DAP	49.8% **	24.3% *	56.0% **	18.6% *

a. *, ** significant difference at 5% and 1% levels, respectively.

There was a highly significant correlation (r^2= 74%) between sweetness and crispness. This suggested that the crispness was related to kernel starch content. The sweeter the kernel, the less is its starch content, and the higher is the crispness. The correlation (r^2 = 24%) between sweetness and pericarp thickness at 18 DAP was significant (P=0.05). This was because the three hybrids with Hawaii-bred parental lines were much sweeter and had a very thin pericarp at 18 DAP. In the absence of the three hybrids, the r^2 value between sweetness and pericarp thickness at 18 DAP was only 3%.

Tenderness mainly consists of pericarp thickness and endosperm texture (Brewbaker, 1977). Ito and Brewbaker (1981) reported that a significant correlation (r^2= 96%) was found between average bite-test scores and immature pericarp thickness for the corn cultivar "Hawaiian Supersweet #9". The correlation between tenderness and pericarp thickness of 18 DAP was highly significant (r^2 = 56%) in this study, but the r^2 value was lower than the one reported by Ito and Brewbaker (1981). This difference confirmed that although pericarp thickness is a major factor affecting tenderness, endosperm texture also influences tenderness. "Hawaiian Supersweet #9" is a cultivar with great sweetness. The sweeter the kernel, the less the starch content and the higher the moisture content. Pericarp thickness appears to affect tenderness more for kernels with higher sweetness

than kernels with lower sweetness. Of the 26 hybrids used in this study, the 10 *su* hybrids and the 7 *bt* hybrids with at least one side parental line from the US mainland had lower sweetness and higher seed weight, which equates to high starch contents. The r^2 value between tenderness and pericarp thickness at 18 DAP, therefore, tended to be lower.

The correlation between crispness and pericarp thickness at 18 DAP was not significant (r^2= 0.19).

5.4 Discussion and summary

5.4.1 Pericarp thickness

Many researchers have proposed that pericarp thickness could be largely influenced by the inner pressure of the corn kernel during the period of kernel development (Randolph, 1936; Wolf *et al*. 1952; Richardson, 1960; Tracy *et al*. 1988).

Based on this hypothesis, it is suggested that corn with different endosperm genes harvested at the same time or with the same endosperm gene but harvested at different time results in a significant difference of dry matter accumulation and moisture content, and thus produces different inner pressures on the pericarp. This inner pressure can greatly influence pericarp thickness.

For kernels harvested at 18 DAP, the + hybrids had a highly significantly thicker pericarp average than the *bt, sh2,* and *su* hybrids. The differences among these three

genes were not significant. The reason of this phenomenon is not clear. It is known that the + hybrids have lower moisture content and therefore, less inner pressure on the pericarp during this stage than their *bt, sh2,* and *su* counterparts. The moisture content must play an important part to determining the inner pressure in this stage since the dry matter accumulation is still at a lower level. Coe and Neuffer (1977) have depicted that the endosperm in *bt, bt2* and *sh2* before drying is like a fluid-filled sac (in *sh2* greatly distended, balloon-like) that develops very little starch.

For kernels harvested at 36 DAP, the *sh2* hybrids had a highly significantly thicker pericarp average than +, *bt,* or *su* hybrids. The differences among these three genotypes were not significant. Similar results have been observed (Helm and Zuber, 1970; Ito, 1980, Juvik, 1992). The explanation for this phenomenon is that among the four endosperm genes, *sh2* has the most severe shrinkage of endosperm at 36 DAP. The average weight of the *sh2* hybrids accounted for only 45.8% of the average weight of their normal counterparts, in comparison to the *bt* hybrids at 62.6% and the *su* hybrids at 81.8%. On the other hand, the moisture content of *sh2* kernels has been drastically decreased during the period from 18 to 36 DAP in Hawaii. The lower quantity of dry matter accumulation and the decreasing moisture content enable *sh2* kernels to have a

lower inner pressure. Groszmann and Sprague (1948) reported that the quantity of pericarp increased with advancing maturity, and the pericarp weights increased somewhat rapidly during the latter stage of kernel development. The inner pressure that decreases at a late stage for *sh2* hybrids may greatly influence the pericarp thickness. For the + hybrids, as the dry matter accumulates gradually, the inner pressure of the kernel reaches its maximum level, the pericarp thickness decreases significantly from 18 DAP to 36 DAP. For *bt* and *su* hybrids, the quantity of dry matter accumulation is higher than for the *sh2* hybrids but lower than the + hybrids. This should create an inner pressure higher than that of *sh2* hybrids during the period from 18 to 36 DAP. The interaction of this higher inner pressure and the increase of pericarp quantity resulted in both *bt* and *su* having a similar pericarp thickness at both 18 DAP and 36 DAP.

Tracy and Schmidt (1987) reported a different change pattern of pericarp thickness at 45 DAP in their study of near-isogenic lines, though they agreed the change of pericarp thickness in part may be due to differential degrees of expansion of the developing endosperm, they found that *Su sh2* with the least seed weight (Schmidt, 1988) was among the lines with thinnest pericarp thickness (38.7 μm), and *Su su2* with heavier seeds had significantly thicker pericarp (48.0 μm). Considering the pericarp thicknesses

they reported were very thin, it could be possible that the increase of pericarp thickness gradually ceased at the stage before *sh2* kernels started to shrink, the greater expansion of *sh2* endosperm resulted in thinner pericarp. If it had not ceased at an earlier stage, the *sh2* kernel with such a thin pericarp at 45 DAP might have much thinner pericarp at the earlier stage and have problem to resist the great inner pressure.

Another finding which supported the hypothesis was that for *sh2* hybrids, pericarp thickness of both germinal and abgerminal sides increased during the period from 18 to 36 DAP, but the rate of increase was very different. The average pericarp thickness for germinal sides and abgerminal sides increased 26.5 μm and 78.5 μm, respectively (Appendix 9). The growth of the germ or the embryo was not influenced by the endosperm gene, the inner pressure on the germinal side, therefore, was subject to less change. This could explain why the increase of pericarp thickness for the *sh2* and *bt* hybrids mainly occurred on the abgerminal sides. In a similar way, the change of pericarp thickness for the *bt,* and *su* hybrids had the same trends.

The highly significant r^2 values between seed weight and the difference of germinal, abgerminal, or average of pericarp thickness harvested at 36 DAP and 18 DAP, also help to prove this hypothesis.

5.4.2 Sensory evaluation

The comparisons of eating quality among *bt, sh2,* and *su* endosperm mutants shows in general, that *su* had the worst and *sh2* had the best eating quality over the three characters: flavor, sweetness and tenderness, although the difference between *bt* and *sh2* was not significant in many cases.

Within *bt* hybrids, the three Hawaii bred *bt* hybrids had much better eating qualities than the other *bt* and *sh2* hybrids whether cooked or not. The extensive genotypic variability for these eating qualities among the *bt* hybrids suggests that the allelic variation at the loci other than *bt* is probably involved. Unfortunately, since there was no inbred line of Hi38*sh2*, only Hi27 X HS can be used to compare the *bt* gene used in Hawaii and *sh2* gene based on the near-isogenic background. When the comparison was not completely based on the near-isogenic background, it was difficult to draw conclusions on the effects of the endosperm gene on flavor, sweetness, tenderness, and crispness.

Pericarp thickness is one of the most important characters that influence flavor and tenderness of supersweet corn. Pericarp thickness appears to affect tenderness more for kernels with higher sweetness than kernels with lower sweetness. Pericarp thickness varied largely among varieties with different genetic background.

Sweetness was influenced mainly by the endosperm mutant gene but the interaction between the endosperm gene and the genetic background of varieties plays an important role.

Since the sweetness of the three Hawaii bred *bt* hybrids have reached the level of commercial use, it can be safely concluded that the eating quality of the *bt*-type supersweet corn can compete with *sh2* type supersweet corn.

CHAPTER 6

CHARACTERS RELATED TO GERMINATION OF SUPERSWEET CORN

6.1 The effects of endosperm genotypes

Many characters that relate to germination of supersweet corn (*bt* and *sh2*) could be affected by the endosperm genotypes. Six characters were considered in this study. These were seed weight, pericarp thickness, bubble volume, seed density, seed conductivity and sweetness. The effects of endosperm genes on these characters were based on 6 *sh2* hybrids and 10 *bt* hybrids since there was no inbred line of Hi38*sh2* to permit an orthogonal set.

6.1.1 Seed weight

Seed weight, which is a function of seed size and density, influences the germination rate of sweet and supersweet corn (Wann, 1980; Andrew, 1982). Seed weights were measured for dry seeds of all 16 hybrids and are summarized in Appendix 12. Three samples of 100 seeds each were taken for each hybrid.

Seed weight averages for the 16 hybrids are summarized in Table 6.1. The data ranged widely from 10.53 to 19.82 gram/100 seeds, with an overall average of 14.21 (Appendix 12). The average weights of the six isogenic hybrids for *bt* and *sh2* were 17.42 and 12.01, respectively, and this difference was highly significant. Comparing *bt* with *sh2*

Table 6.1 Seed weight for bt vs. sh2 supersweet corn (gram/100 seeds)

Hybrid	*bt* Avg	*sh2* Avg	Differences (bt - sh2)
Hi27 X B37	17.06	11.10	5.96
Hi27 X Oh43	18.39	11.50	6.89
Oh43 X B37	18.81	10.82	7.99
HS X Hi27	12.25	12.95	-0.70
HS X B37	18.44	12.37	6.07
HS X Oh43	19.59	13.29	6.29
Hi38 X Hi27	12.80		
Hi38 X B37	16.59		
Hi38 X Oh43	16.75		
Hi38 X HS	13.30		
Avg (With Hi38)	**16.40**		
Avg (Without Hi38)	**17.42**	**12.01**	**5.42**

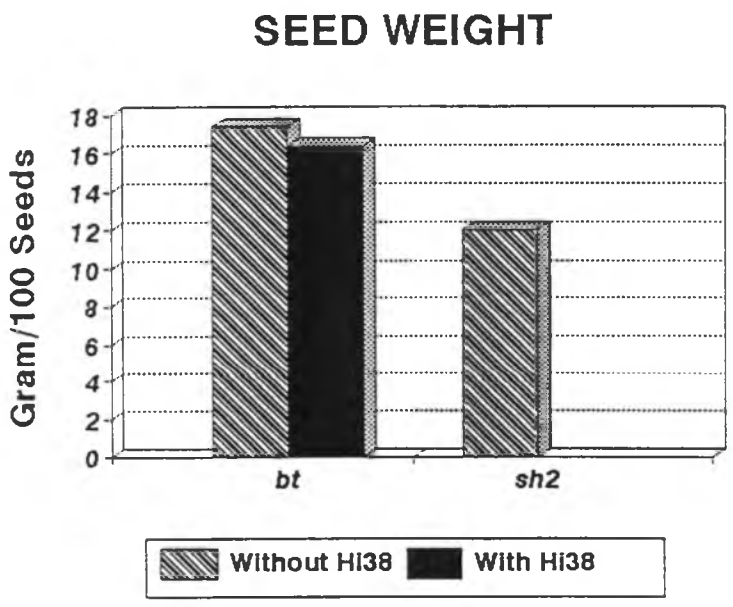

Figure 6.1 Average seed weights for bt and sh2 hybrids

seeds of the same genotype, the *bt* hybrid usually had much heavier seed than its *sh2* counterpart. One exception to this trend was the hybrid HS X Hi27 in which little difference was observed.

The significant comparison in seed weight of the two supersweet genotypes is evident in Figure 6.1. Averages are given for all 10 *bt* hybrids (16.40) and for the 6 hybrids without Hi38 (17.42) having counterpart *sh2* types (12.01).

Analyses of variance (Table 6.2) confirmed the significance of differences between all genotypes of the *bt* and *sh2* hybrids. The variances among hybrids within genotypes were also highly significant. Error variances were very low, resulting in CV's of 4.65% and 4.29%. Averages and LSDs are presented in this table.

6.1.2 Pericarp thickness

Pericarp thickness is one of the most important components of supersweet corn eating quality. It is also a very important character which influences germination of supersweet corn through its effect on bubble volume, according to the hypothesis proposed in this study.

Pericarp thicknesses were measured 36 days after pollination for dry seeds of all 16 hybrids and are summarized in Appendix 8. Methods for assessing pericarp thickness (in microns) were described in Section 3.2.1. Samples of ten seeds were taken from each hybrid.

Table 6.2 Analysis of variance of seed weight for bt vs. sh2 supersweet corn (gram/100 seeds)

ANOV: (Without HI38)

Source	df	SS	MS	F		F.05	F.01
Between Genotypes	1	264.26	264.26	21.68	**	4.96	10.0
Betw. Variety within Geno.	10	121.92	12.19	26.03	**	2.26	3.17
Error (Among Varieties)	24	11.24	0.47				
Total	35	397.41					

CV =	4.65%	Genotypes	Average	
		bt	17.42	g/100 seeds
LSD.05 =	2.59	*sh2*	12.01	g/100 seeds
LSD.01 =	3.69	Difference	5.42	g/100 seeds

ANOV: (With HI38)

Source	df	SS	MS	F		F.05	F.01
Between Genotypes	1	217.18	217.18	14.55	**	4.60	8.86
Betw. Variety within Geno.	14	209.00	14.93	37.35	**	2.02	2.70
Error (Among Varieties)	32	12.79	0.40				
Total	47	438.96					

CV =	4.29%	Genotypes	Average	
		bt	16.40	g/100 seeds
LSD.05 =	2.39	*sh2*	12.01	g/100 seeds
LSD.01 =	3.32	Difference	4.39	g/100 seeds

Averages for pericarp thickness are summarized in Table 6.3. The data ranged widely from 61.4 μm to 159.8 μm, with an overall average of 108.9 μm (Appendix 8). The average thicknesses for *bt* and *sh2* seeds, based on the six isogenic hybrids, were 106 μm and 139.7 μm, respectively, and this difference was highly significant.

Comparing *bt* with *sh2* seeds of the same genotype, in general, there were large differences. All *sh2* hybrids had thicker pericarps than their *bt* counterparts, with some differences exceeding 40% of the thick genotype. An exception for this comparison was the hybrid HS X Hi27, in which the difference was relatively small (11.3 μm). For HS X Hi27, both *bt* and *sh2* counterparts had similar seed weights (referred to later in Table 6.15), therefore, there could be similar kernel inner pressure on the pericarp, which resulted in the similar pericarp thickness. The largest difference of pericarp thickness (60.1 μm) was observed between *bt* and *sh2* counterparts of the hybrid HS X Oh43. The thinner pericarp of HS*bt* X Oh43*bt* could be partly due to the much heavier seed weight of this hybrid, which resulted in a greater inner pressure during seed development (Table 6.15).

The significant comparison in pericarp thickness of the two supersweet genotypes is evident in Figure 6.2. Averages are given for all 10 *bt* hybrids (96.0) and for the 6 hybrids without Hi38 (106.0) having counterpart *sh2* types (139.7).

Table 6.3 Pericarp thickness at 36 days after pollination for bt vs. sh2 supersweet corn (microns)

Hybrid	*bt* Avg	*sh2* Avg	Differenc (sh2 - bt)	Parental lines	*bt* Avg	*sh2* Avg
Hi27 X B37	128.0	159.8	31.8	Hi27	85.9	108.3
Hi27 X Oh43	109.0	141.7	32.7	HS	68.3	87.6
Oh43 X B37	121.9	137.3	15.4	B37	179.2	182.2
HS X Hi27	99.7	111.0	11.3	Oh43	105.7	129.4
HS X B37	108.0	159.0	51.0	Hi38	58.2	
HS X Oh43	69.3	129.4	60.1			
Hi38 X Hi27	81.9					
Hi38 X B37	98.3					
Hi38 X Oh43	82.9					
Hi38 X HS	61.4					
Avg (With Hi38)	**96.0**				**99.5**	
Avg (Without Hi38)	**106.0**	**139.7**	**33.7**		**109.7**	**126.9**

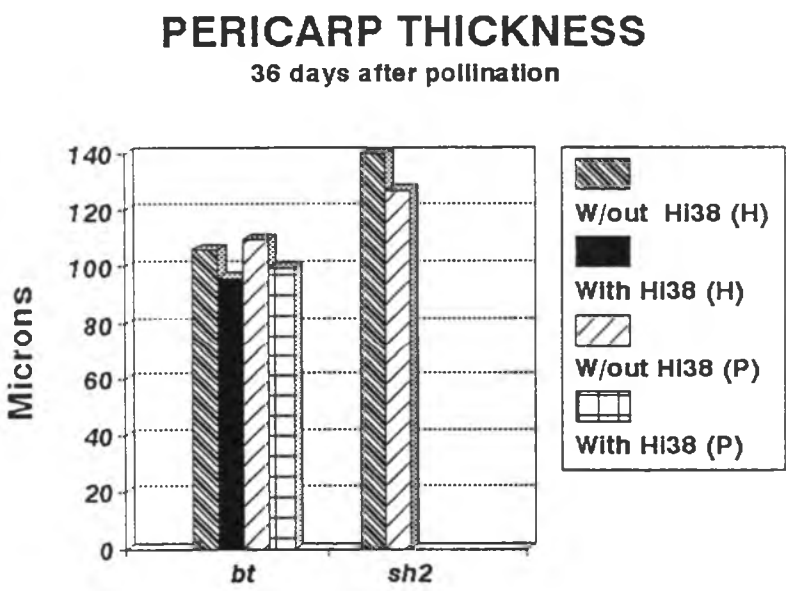

Figure 6.2 Average pericarp thickness for bt and sh2 hybrids (H) and their parental lines (P)

Analyses of variance (Table 6.4) confirmed the significance of differences between all genotypes of both the *bt* and *sh2* hybrids. The variances among hybrids within genotypes were also highly significant. Error variances were relatively high, resulting in CV's of 13.4% and 13.3%. Averages and LSDs are presented in this table. The F value was significant when comparing *bt* with *sh2* seeds of the same genotype. The difference between *bt* and *sh2* was larger than the value of LSD 0.01.

The average pericarp thickness of 108.9 μm was considered to be very thick. It was mainly caused by the inbred lines of B37, Hi27, and Oh43 series, all of which have very thick pericarps (Appendix 6).

The phenomenon that *sh2* endosperm is associated with thick pericarp was also evident in the parental lines of these hybrids (Figure 6.2).

6.1.3 Bubble volume

Bubble volume is the separation of endosperm from pericarp and is a very common phenomenon in supersweet corn seed. The volume of bubbles could influence seed germination adversely by causing the pericarp to crack easily with more severe imbibition damage and soil-borne fungi attacks (Styer *et al.*, 1983; Wenn, 1986; Chern *et al.*, 1991; Douglass *et al.*, 1993).

Bubble volume was quantified for dry seeds of all 16

Table 6.4 Analysis of variance of pericarp thickness at 36 DAP for bt vs. sh2 supersweet corn (microns)

ANOV: (Without Hi38)

Source	DF	SS	MS	F		F.05	F.01
Between Genotypes	1	34104	34104.4	8.86	*	4.96	10.0
Betw. Variety within Geno.	10	38476	3847.6	14.12	**	1.92	2.51
Error (Among Varieties)	108	29426	272.5				
Total	119	102006					

CV =	13.44%	Genotypes	Average	
		bt	106.0	microns
LSD.05 =	25.23	*sh2*	139.7	microns
LSD.01 =	35.89	Difference	33.7	microns

ANOV: (With Hi38)

Source	DF	SS	MS	F		F.05	F.01
Between Genotypes	1	71499	71498.7	16.63	**	4.60	8.86
Betw. Variety within Geno.	14	60200	4300.00	19.12	**	1.76	2.20
Error (Among Varieties)	144	32385	224.90				
Total	159	164084					

CV =	13.34%	Genotypes	Average	
		bt	96.0	microns
LSD.05 =	22.24	*sh2*	139.7	microns
LSD.01 =	30.866	Difference	43.7	microns

hybrids and summarized in Appendix 10. Methods for assessing bubble volume (in ml per 100 seeds) were described in Section 3.3.1. Three samples were taken of 10 seeds for each hybrid.

Bubble volume averages for the 16 hybrids are summarized in Table 6.5. The data ranged widely from 0.33 ml/100 seeds to 2.97 ml/100 seeds, with an overall average of 1.73 (Appendix 10). The average volume, based on the six isogenic hybrids for *bt* and *sh2* seeds, were 1.33 and 2.74, respectively, and this difference was highly significant. Comparing *bt* with *sh2* seeds of the same genotype, there was approximately a doubling of bubble volume. One exception to this comparison was the hybrid HS X Hi27, in which little difference could be observed. This appears to result from the fact that HS*bt* X Hi27*bt* and HS*sh2* X Hi27*Sh2* differed little in pericarp thickness and seed weight (referred to later in Table 6.15). According to the hypothesis proposed in this study, bubble volume is determined by both pericarp thickness and shrinkage of endosperm.

The significant comparison in bubble volume of the two supersweet genotypes is evident in Figure 6.3. Averages are given for all 10 *bt* hybrids (1.13) and for the 6 hybrids with Hi38 (1.33) with counterpart *sh2* types (2.74). The large difference between the averages of *bt* and *sh2* genotypes may be attributed to the joint effect of pericarp thickness and shrinkage of endosperm.

Table 6.5 Bubble volume for bt vs. sh2 supersweet corn (ml/100 seeds)

Hybrid	*bt* Avg	*sh2* Avg	Differences (sh2 - bt)
Hi27 X B37	2.00	2.97	0.97
Hi27 X Oh43	1.37	2.27	0.90
Oh43 X B37	0.67	3.80	3.13
HS X Hi27	1.77	1.83	0.07
HS X B37	1.13	2.07	0.93
HS X Oh43	1.03	3.50	2.47
Hi38 X Hi27	1.50		
Hi38 X B37	0.63		
Hi38 X Oh43	0.83		
Hi38 X HS	0.33		
Avg (With Hi38)	**1.13**		
Avg (Without Hi38)	**1.33**	**2.74**	**1.41**

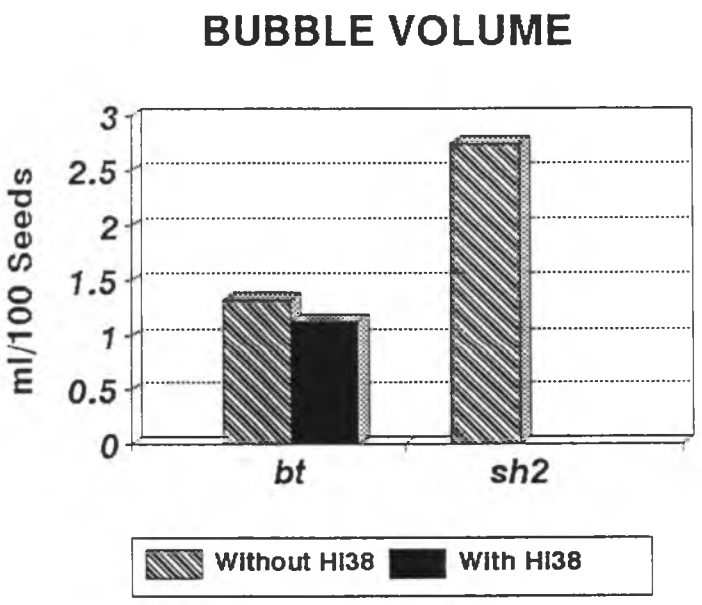

Figure 6.3 Average bubble volumes for bt and sh2 hybrids

Analyses of variance (Table 6.6) confirmed the significance of differences between all genotypes of both the *bt* and *sh2* hybrids. The variances among hybrids within genotypes were also highly significant. Error variances were relatively high, resulting in CV's of 18% and 20%. Averages and LSDs are also summarized in the Table.

6.1.4 Seed Density

Density is usually expressed as specific gravity in comparison with water (specific gravity of 1.0). Seed density of supersweet corn is influenced mainly by bubble volume which affects germination; the higher the bubble volume, the lower the seed density (assuming constant moisture contents).

Seed densities were quantified for dry seeds of all 16 hybrids and summarized in Appendix 11. Methods for assessing seed density (in gram per ml) were described in Section 3.3.2. Three samples of 25 seeds each were taken for each hybrid.

Seed density averages for the 16 hybrids are summarized in Table 6.7. The data ranged widely from 0.87 g/ml to 1.2 g/ml, with an overall average of 1.02 (Appendix 11). The average densities, based on the six isogenic hybrids for *bt* and *sh2* seeds, were 1.12 and 0.9, respectively, and this difference was highly significant. Comparing *bt* with *sh2* seeds of the same genotype, there was often a large

Table 6.6 Analysis of variance of bubble volume for bt vs. sh2 supersweet corn (ml/100 seeds)

ANOVA: (Without HI38)

Source	df	SS	MS	F		F.05	F.01
Between Genotypes	1	17.92	17.92	13.40	**	4.96	10.0
Betw. Variety within Geno.	10	13.38	1.34	10.36	**	2.26	3.17
Error (Among Varieties)	24	3.10	0.13				
Total	35	34.40					

CV =	17.68%	Genotypes	Average	
		bt	1.33	ml/100 seeds
LSD.05 =	0.86	*sh2*	2.74	ml/100 seeds
LSD.01 =	1.22	Difference	1.41	ml/100 seeds

ANOVA: (With Hi38)

Source	df	SS	MS	F		F.05	F.01
Between Genotypes	1	29.24	29.24	23.53	**	4.60	8.86
Betw. Variety within Geno.	14	17.40	1.24	10.30	**	2.02	2.70
Error (Among Varieties)	32	3.86	0.12				
Total	47	50.50					

CV =	20.06%	Genotypes	Average	
		bt	1.13	ml/100 seeds
LSD.05 =	0.69	*sh2*	2.74	ml/100 seeds
LSD.01 =	0.96	Difference	1.61	ml/100 seeds

Table 6.7 Seed density for bt vs. sh2 supersweet corn (gram/ml)

Hybrid	*bt* Avg	*sh2* Avg	Differences (bt - sh2)
Hi27 X B37	0.98	0.87	0.12
Hi27 X Oh43	1.09	0.88	0.20
Oh43 X B37	1.33	0.85	0.48
HS X Hi27	1.04	0.98	0.05
HS X B37	1.16	0.92	0.24
HS X Oh43	1.12	0.92	0.21
Hi38 X Hi27	1.07		
Hi38 X B37	1.18		
Hi38 X Oh43	1.16		
Hi38 X HS	1.20		
Avg (With Hi38)	**1.13**		
Avg (Without Hi38)	1.12	**0.90**	**0.22**

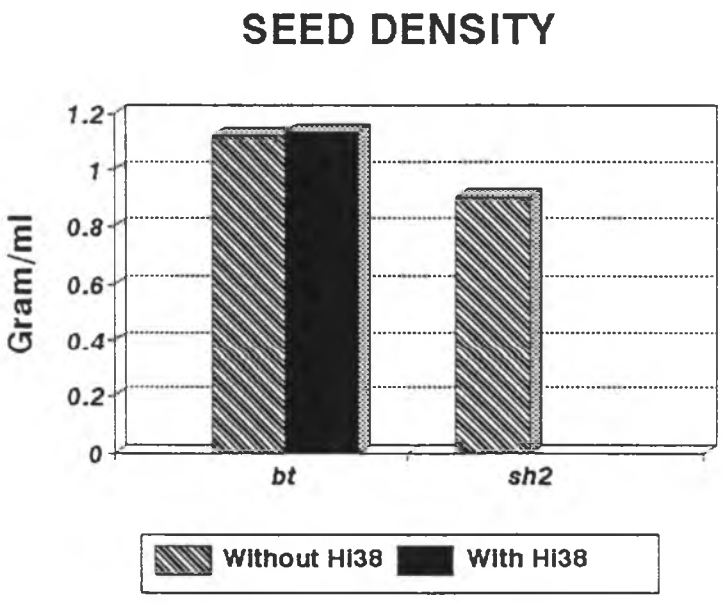

Figure 6.4 Average seed densities for bt and sh2 hybrids

difference of seed density between the two endosperm types. One exception to this comparison was the hybrid HS X Hi27, in which little difference could be observed. This was because HS*bt* X Hi27*bt* and HS*sh2* X Hi27*sh2* differed little in bubble volume which exerted a major influence on seed density of supersweet corn (referred to later in Table 6.15).

The significant comparison in seed density of the two supersweet genotypes is evident in Figure 6.4. Averages are given for all 10 *bt* hybrids (1.13) and for the 6 hybrids without Hi38 (1.12) having counterpart *sh2* types (0.90).

Analyses of variance (Table 6.8) confirmed the significance of differences between all genotypes of the *bt* and *sh2* hybrids. The variances among hybrids within genotypes were also highly significant. Error variances were relatively low, resulting in CV's of 8.1% and 7.11%. Averages and LSDs are presented in this Table.

6.1.5 Seed conductivity:

The effect of endosperm gene on seed conductivity or seed leachate electrolyte conductivity has been reported by many researchers (Styer and Cantliffe, 1983; Tracy *et al.*, 1988). Studies involving endosperm with *bt* gene have not been reported. In published studies, *sh2* seeds usually had the highest conductivity in comparison to the other endosperm mutants. Conductivity was negatively and highly

Table 6.8 Analysis of variance of seed density for bt vs. sh2 supersweet corn (gram/ml)

ANOV: (Without Hi38)

Source	df	SS	MS	F		F.05	F.01
Between Genotypes	1	0.43	0.43	17.07	**	4.96	10.0
Betw. Variety within Geno.	10	0.25	0.02	3.72	**	2.26	3.17
Error (Among Varieties)	24	0.16	0.01				
Total	35	0.84					

CV =	8.10%	Genotypes	Average	
		bt	1.12	g/ml
LSD.05 =	0.12	*sh2*	0.90	g/ml
LSD.01 =	0.17	Difference	0.22	g/ml

ANOV: (With Hi38)

Source	df	SS	MS	F		F.05	F.01
Between Genotypes	1	0.60	0.60	29.09	**	4.60	8.86
Betw. Variety within Geno.	14	0.29	0.02	3.71	**	2.02	2.70
Error (Among Varieties)	32	0.18	0.01				
Total	47	1.06					

CV =	7.11%	Genotypes	Average	
		bt	1.13	g/ml
LSD.05 =	0.09	*sh2*	0.90	g/ml
LSD.01 =	0.12	Difference	0.23	g/ml

correlated with germination rates.

The seed conductivities were measured for dry seeds of all 16 hybrids and summarized in Appendix 2. Methods for assessing seed conductivity (in milli Siemens/meter) were described in Section 3.1.5. Three samples were taken of 10 seeds for each hybrid.

Seed conductivity averages for the 16 hybrids are summarized in Table 6.9. The data ranged widely from 10.49 to 32.80 milli S/m, with an overall average of 22.46 (Appendix 2). The average seed conductivities, based on the six isogenic hybrids for *bt* and *sh2* seeds, were 20.23 and 27.24, respectively, and this difference was significant.

The significant comparison in seed conductivity of the two supersweet genotypes is evident in Figure 6.5. Averages are given for all 10 *bt* hybrids (17.68) and for the 6 hybrids without Hi38 (20.23) having counterpart *sh2* types (27.24).

Analyses of variance (Table 6.10) confirmed the significance of differences between all genotypes of the *bt* and *sh2* hybrids. The variances among hybrids within genotypes were also highly significant. Error variances were relatively high, resulting in CV's of 12.18% and 12.68%. Averages and LSDs are presented in this table.

6.1.6 Sweetness

Sweetness is one of the most important factors

Table 6.9 Seed leachate conductivity for bt vs. sh2 supersweet corn (milli Siemens/meter)

Hybrid	*bt* Avg	*sh2* Avg	Differences (sh2 - bt)
Hi27 X B37	19.36	29.53	10.17
Hi27 X Oh43	25.57	29.83	4.27
Oh43 X B37	21.83	32.80	10.97
HS X Hi27	18.44	18.19	-0.25
HS X B37	17.22	29.53	12.32
HS X Oh43	18.94	23.57	4.63
Hi38 X Hi27	10.49		
Hi38 X B37	15.70		
Hi38 X Oh43	17.32		
Hi38 X HS	11.95		
Avg (With Hi38)	**17.68**		
Avg (Without Hi38)	**20.23**	**27.24**	**7.02**

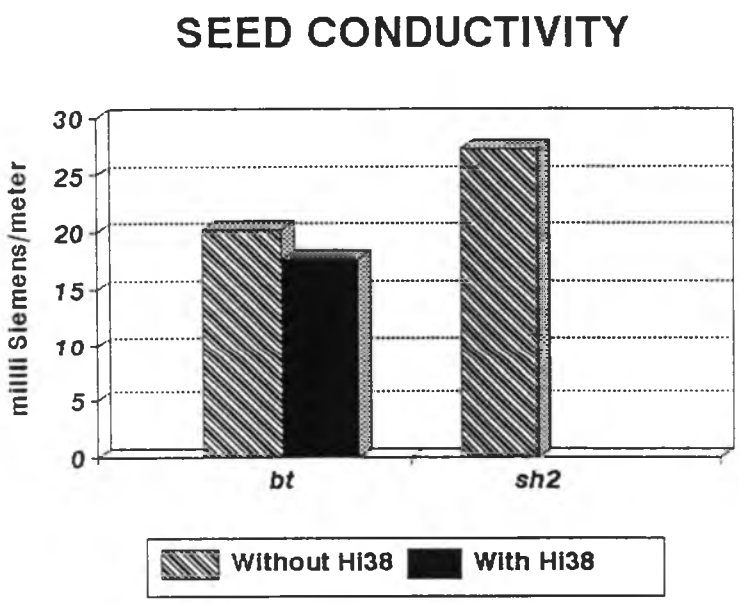

Figure 6.5 Average seed leachate conductivities for bt and sh2 hybrids

Table 6.10 Analysis of variance of seed conductivity for bt vs. sh2 supersweet corn (milli Siemens/meter)

ANOVA: (Without Hi38)

Source	df	SS	MS	F		F.05	F.01
Between Genotypes	1	443.10	443.10	7.80	*	4.96	10.0
Betw. Variety within Geno.	10	567.72	56.77	6.79	**	2.26	3.17
Error (Among Varieties)	24	200.54	8.36				
Total	35	1211.4					

CV =	12.18%	Genotypes	Average	
		bt	20.23	milli S/m
LSD.05 =	5.60	*sh2*	27.24	milli S/m
LSD.01 =	7.96	Difference	7.02	milli S/m

ANOVA: (With Hi38)

Source	df	SS	MS	F		F.05	F.01
Between Genotypes	1	1028.6	1028.61	15.15	**	4.60	8.86
Betw. Variety within Geno.	14	950.4	67.88	9.34	**	2.02	2.70
Error (Among Varieties)	32	232.7	7.27				
Total	47	2211.6					

CV =	12.68%	Genotypes	Average	
		bt	17.68	milli S/m
LSD.05 =	5.10	*sh2*	27.24	milli S/m
LSD.01 =	7.08	Difference	9.56	milli S/m

affecting the eating quality of sweet and supersweet corn. Sweetness has been known to adversely affect the germination rates of both sweet and supersweet corn.

Sweetness was evaluated for fresh ears at 18 days after pollination of all 16 hybrids and summarized in Appendix 15. Methods for assessing sweetness (in a 1 to 9 hedonic scale) were described in Section 3.2.2. Three samples of each hybrid were taken to create averages of cooked and fresh bite tests.

Sweetness averages for the 16 hybrids are summarized in Table 6.11. The data ranged widely from 1.72 to 6.94, with an overall average of 4.39 (Appendix 15). The average sweetness based on the six isogenic hybrids for *bt* and *sh2* seeds were 5.48 and 3.74, respectively, and this difference was at the 5% significance level. The *bt* hybrid was usually inferior in sweetness to the *sh2* hybrid of the same genotype. One exception observed was the hybrid HS*bt* X Hi27*bt* which had superior sweetness relative to its *sh2* counterpart. The extensive genotypic variability for sweetness among the *bt* varieties indicated that allelic variation at loci other than *bt* is involved.

The significant comparison in sweetness of the two supersweet genotypes is evident in Figure 6.6. Averages are given for all 10 *bt* hybrids (5.03) and for the 6 hybrids without Hi38 (5.48) having counterpart *sh2* types (3.74).

Analyses of variance (Table 6.12) confirmed the

Table 6.11 Sweetness for bt vs. sh2 supersweet corn on a 1 - 9 scale (1= the best, 9=the worst)

Hybrid	*bt* Avg	*sh2* Avg	Differences (bt - sh2)
Hi27 X B37	5.64	4.03	1.61
Hi27 X Oh43	5.56	4.14	1.42
Oh43 X B37	6.14	3.64	2.50
HS X Hi27	2.33	4.19	-1.86
HS X B37	6.25	2.75	3.50
HS X Oh43	6.94	3.69	3.25
Hi38 X Hi27	3.75		
Hi38 X B37	6.03		
Hi38 X Oh43	5.89		
Hi38 X HS	1.72		
Avg (With Hi38)	**5.03**		
Avg (Without Hi38)	**5.48**	**3.74**	**1.74**

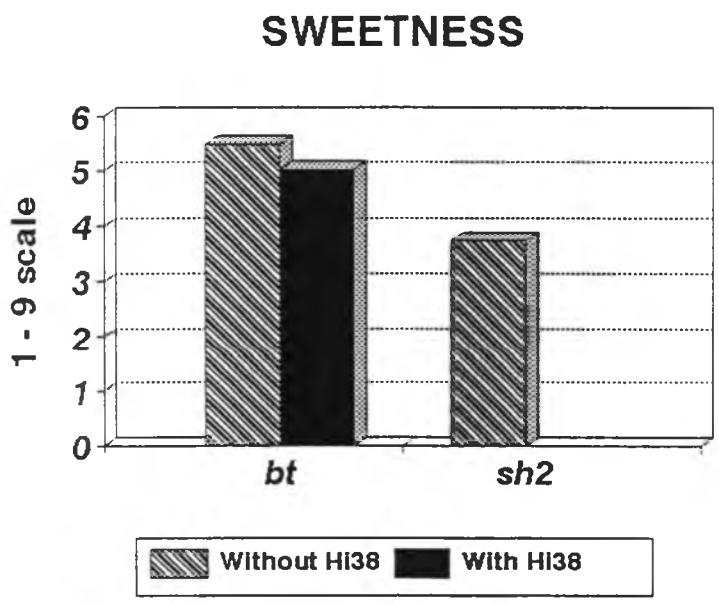

Figure 6.6 Average sweetness for bt and sh2 hybrids

Table 6.12 Analysis of variance of sweetness for bt vs. sh2 supersweet corn on a 1 - 9 scale

ANOV: (Without Hi38)

Source	df	SS	MS	F		F.05	F.01
Between Genotypes	1	27.13	27.13	6.22	*	4.96	10.0
Betw. Variety within Geno.	10	43.63	4.36	35.73	**	2.26	3.17
Error (Among Varieties)	24	2.93	0.12				
Total	35	73.69					

CV =	7.58%	Genotypes	Average
		bt	5.48
LSD.05 =	1.55	*sh2*	3.74
LSD.01 =	2.21	Difference	1.74

ANOV: (With Hi38)

Source	df	SS	MS	F		F.05	F.01
Between Genotypes	1	18.55	18.55	2.88		4.60	8.86
Betw. Variety within Geno.	14	90.17	6.44	60.82	**	2.02	2.70
Error (Among Varieties)	32	3.39	0.11				
Total	47	112.11					

CV =	7.16%	Genotypes	Average
		bt	5.03
LSD.05 =	1.57	*sh2*	3.74
LSD.01 =	2.18	Difference	1.28

significance of differences between all genotypes of the *bt* and *sh2* hybrids. The variances among hybrids within genotypes were also highly significant. Error variances were very low, resulting in CV's of 7.58% and 7.16%. Averages and LSDs are presented in this table.

6.2 Correlations

The relationships among the five different germination tests and the six different germination related characters were estimated by coefficients of determination (r^2) and presented in Table 6.13, and discussed in detail in the following sections. Among the five different germination tests, the cold soil germination test had the lowest r^2 values. These lower r^2 values are attributed mainly to the excessive moisture content of the absorbent paper towel, since even the hybrids with normal endosperm had much lower germination rates in this test (Appendix 4).

The correlations among the six different germination related characters, estimated by r^2, were presented in Table 6.14 and discussed in the following sections.

Combined germination rate and combined conductivity were used to simplify the resulting statements since similar relationships were found among the hybrids and test variables. The combined germination rate was the average from seven different germination tests and the combined conductivity was the average from three conductivity tests.

Table 6.13 Coefficients of determination among different germination tests and the 6 characters (bt and sh2 only)

Germinatio tests	Pericarp Thickness	Bubble volume	Seed density	Seed weight	Conductivity	Sweetness
	(-)	(-)	(+)	(+)	(-)	(+)
A	55.9% **	53.9% **	57.8% **	36.8% *	58.3% **	9.9% ns
B	75.3% **	40.9% **	41.5% **	31.4% *	73.2% **	7.7% ns
C	81.5% **	64.8% **	64.3% **	27.1% *	77.3% **	3.1% ns
D	71.4% **	56.0% **	60.8% **	17.2% ns	62.9% **	0.6% ns
E	49.2% **	30.8% *	27.3% *	11.6% ns	57.4% **	6.5% ns

a. A = Standard germination, B = Cold germination, C = AA warm germination (without drying), D = AA warm germination (after drying), E = Cold soil germination (1 soil : 2 sand).

b. (-) means that the correlation is negative, (+) means that the correlations is positive.

c. *, ** Significant differences at the 5% and 1% levels, respectively.

Table 6.14 Coefficients of determination among the various characters (bt and sh2 only)

Characters	CG	CC	BV	SD	PT	SW
CC (-)	85.8% **					
BV (-)	61.5% ** (+)	50.6% **				
SD (+)	60.3% ** (-)	45.7% ** (-)	81.0% **			
PT (-)	81.1% ** (+)	78.2% ** (+)	49.8% ** (-)	45.8% **		
SW (+)	30.3% * (-)	18.8% ns (-)	40.7% ** (+)	53.7% ** (-)	18.1% ns	
SWS (+)	6.4% ns (-)	3.8% ns (-)	11.7% ns (+)	18.7% ns (-)	2.2% ns (+)	70.1% **

a. CG = Combined germination, it is an average from the 5 different germination tests.
CC = Combined conductivity, it is an average from three different conductivity tests.
BV = Bubble volume
SD = Seed density
PT = Pericarp thickness
SW = Seed weight
SWS = Sweetness

b. *, ** Significant differences at the 5% and 1% levels, respectively.

6.2.1 Seed weight

The correlations among seed weight and three of the five different germination tests were significant (P = 0.05) (Table 6.13).

The correlations between seed weight and the other five germination related characters as well as the combined germination rate are presented in Table 6.14. The correlations (r^2) of seed weight with bubble volume (40.7%) and seed density (53.7%) were highly significant, since seed weight is a function of density and size. The correlation between seed weight and combined germination (30.3%) was significant at the 5% level.

6.2.2 Pericarp thickness

The correlations between pericarp thickness and the five different germination tests were highly significant (P = 0.01) (Table 6.13), although the correlation between pericarp thickness and the cold soil test had a relatively lower r^2 value. As pericarp thickness increased, germination decreased.

The correlations between pericarp thickness and the other five germination-related characters as well as the combined germination rate are presented in Table 6.14. The correlation (r^2) of pericarp thickness with the combined germination rate (81.1%) and combined conductivity (78.2%) were among the highest.

According to the hypothesis proposed in this study, pericarp thickness influences the germination rate via the formation of bubble volume, which increases with increasing pericarp thickness and increasing shrinkage of the endosperm (reducing seed density). A question here was why the r^2 value of the combined germination rate with pericarp thickness (81.1%) was much higher than the one with bubble volume (61.5%) (Table 6.14). The pericarp thickness and seed weight were negatively correlated ($r = -0.43$) in this study. The correlation of germination rate with pericarp thickness must be influenced by this negative correlation. A path analysis was conducted to analyze the relationships among pericarp thickness, seed weight, and combined germination rate. It was found that the direct effect of pericarp thickness on combined germination rate was -0.81 (R^2 = 66.4%), which was close to the r^2 value of bubble volume with combined germination rate (61.5%).

6.2.3 Bubble volume

The correlations between bubble volume and the four different germination tests were highly significant ($P = 0.01$), while the correlation was at the $P = 0.05$ level for the cold soil test (Table 6.13). As bubble volume increased, germination rate dropped.

The correlations between bubble volume and the other five germination-related characters were presented in Table

6.14. The coefficients of determination of bubble volume with combined germination rate (61.5%) and combined conductivity (50.6%) were, as expected, highly significant (P = 0.01). The relationship between bubble volume and combined conductivity is shown in Figure 6.7. Bubble volume is mainly determined by pericarp thickness and shrinkage of endosperm according to the hypothesis proposed in this study. The r^2 of the simple linear correlation of bubble volume with pericarp thickness and seed weight were 49.8% and 40.7%, respectively (Table 6.14). Both were highly significant (P = 0.01). The relationship between pericarp thickness and bubble volume is evident in Figure 6.8.

6.2.4 Seed density

The correlations between seed density and the first four different germination tests were highly significant (P = 0.01), while the correlation was at the P = 0.5 level for the cold soil germination test (Table 6.13). As seed density decreased, germination rates dropped.

The correlations (r^2) between seed density and the other five germination-related characters were presented at Table 6.14. The correlation between seed density and bubble volume had a very high r^2 value of 81.0%, which suggested that bubble volume was highly influenced by seed density for supersweet corn. For this reason, the correlations of seed density with pericarp thickness and seed weight were 45.8%

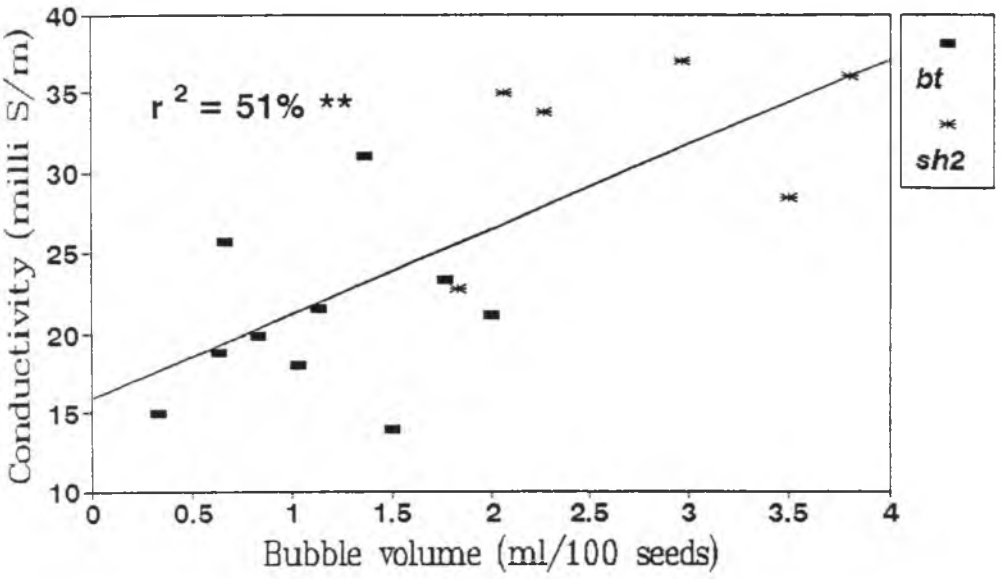

Figure 6.7 Correlation between bubble volume and (combined) conductivity

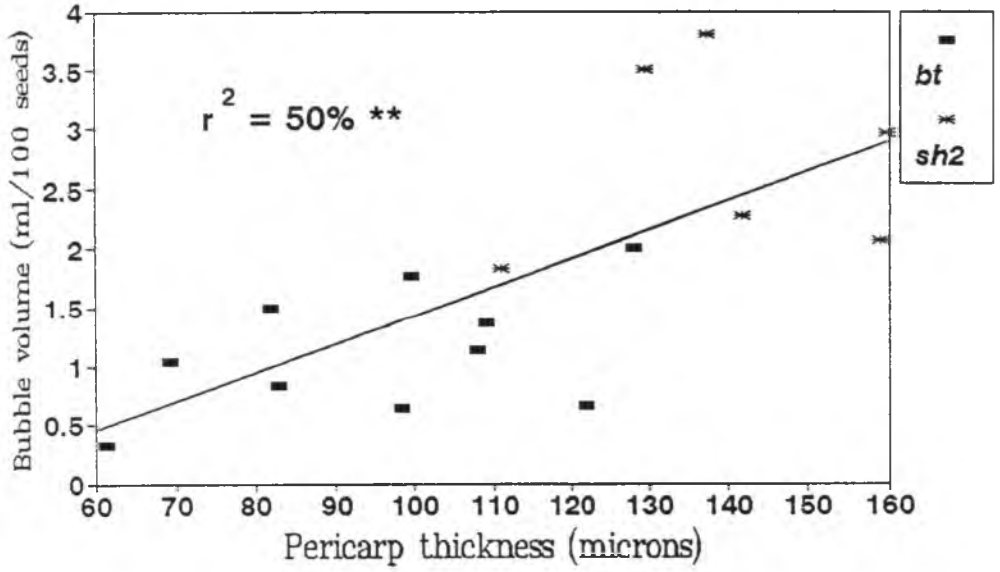

Figure 6.8 Correlation between pericarp thickness and bubble volume

and 53.7%, respectively. Seed density, similar to bubble volume, also had highly significant r^2 values with combined germination rate (60.3%) and combined conductivity (45.7%) (Table 6.14).

6.2.5 Seed conductivity

The correlations between seed leachate conductivity and the five different germination tests were highly significant (P = 0.01) (Table 6.13). As conductivity increased, germination decreased.

The correlations between seed conductivity with the five germination-related characters and combined germination rate are presented in Table 6.14. The r^2 value between combined conductivity and combined germination rate was 85.8%.

The correlation between seed conductivity and seed germination both in the lab and field has been reported by many researchers. Our study confirmed their results.

The correlations between seed conductivity and bubble volume, seed density, and pericarp thickness were all highly significant (Table 6.14).

6.2.6 Sweetness

The correlations between sweetness and the five different germination tests were not significant (Table 6.13).

The correlations between sweetness and the other five germination related characters are presented in Table 6.14. The correlation (r^2) of sweetness with seed weight was the only highly significant one (70.1%), while the others were not significant. The sugar contents of both the *bt* and *sh2* genotypes increase greatly by hindering the conversion of sucrose to the substrates for starch synthesis. The observed high negative correlation between seed weight and sweetness was expected.

6.3 Discussion and summary

6.3.1 The effects of endosperm genotypes on measured traits

There were significant differences, in general, for all of the six characters when comparing isogenic *bt* and *sh2* hybrids. These differences could be attributed largely to the same reason, that is, the effects of *bt* or *sh2* gene hinder the conversion of sucrose to the substrates for starch synthesis. In other words, the shrinkage of endosperm mediated by the *bt* or *sh2* gene could be the primary reason of those differences. The *bt* hybrids usually had less shrinkage of endosperm than their *sh2* counterparts, with the exception of HS*bt* X Hi27*bt* which consisted of only Hawaii-bred lines and had small differences from its *sh2* counterpart for all six characters (Table 6.15). The extensive genotypic variability for seed weight and consequently for the other five characters among the *bt*

Table 6.15 Summary of the 6 germination-related characters for the 6 isogenic hybrids

Hybrids		Seed weight (g/100 seeds)	PT (micron	Bubble volume (ml/100 seeds)	Seed density (g/ml)	Con-ductivity (milii S/m)	Sweet-ness (1 - 9 scale)
Hi27 X B37	*bt*	17.1	128.0	2.00	0.98	19.36	5.64
	sh2	11.1	159.8	2.97	0.87	29.53	4.03
Diff. (absolute value)		6.0	31.8	0.97	0.12	10.17	1.61
Hi27 X Oh43	*bt*	18.4	109.0	1.37	1.09	25.57	5.56
	sh2	11.5	141.7	2.27	0.88	29.83	4.14
Diff. (absolute value)		6.9	32.7	0.90	0.20	4.27	1.42
Oh43 X B37	*bt*	18.8	121.9	0.67	1.33	21.83	6.14
	sh2	10.8	137.3	3.80	0.85	32.80	3.64
Diff. (absolute value)		8.0	15.4	3.13	0.48	10.97	2.50
HS X Hi27	*bt*	12.3	99.7	1.77	1.04	18.44	2.33
	sh2	13.0	111.0	1.83	0.98	18.19	4.19
Diff. (absolute value)		0.7	11.3	0.07	0.05	0.25	1.86
HS X B37	*bt*	18.4	108.0	1.13	1.16	17.22	6.25
	sh2	12.4	159.0	2.07	0.92	29.53	2.75
Diff. (absolute value)		6.1	51.0	0.93	0.24	12.32	3.50
HS X Oh43	*bt*	19.6	69.3	1.03	1.12	18.94	6.94
	sh2	13.3	129.4	3.50	0.92	23.57	3.69
Diff. (absolute value)		6.3	60.1	2.47	0.21	4.63	3.25

hybrids indicated that allelic variation at loci other than *bt* is probably involved.

Seed weight is a function of seed density and seed size. For supersweet corn, the shrinkage of endosperm greatly influences seed density and seed size.

Pericarp thickness is controlled genetically but is influenced greatly by inner pressure during kernel development. This morphological phenomenon was discussed in Chapter 5.1. The shrinkage of endosperm may be one of the main factors to determine the kernel inner pressure. The *sh2* hybrids with very shrunken endosperm had significantly thicker pericarps than their *bt* counterparts. Similar results have been observed by Ito (1980).

Bubble volume is determined mainly by shrinkage of endosperm and pericarp thickness. Although there was a highly significant difference of bubble volume between *bt* and *sh2* genotypes in this study, it should not be attributed only to the effects of *bt* and *sh2* endosperm genes. The genetic background of corn itself plays a more important role in determining the pericarp thickness, although the shrinkage of endosperm can influence pericarp thickness. Kernels with thin pericarp tend to have little bubble volume, whether the shrinkage of endosperm is substantial or not, whereas kernels with thick pericarp and higher shrinkage of endosperm tend to have large bubble volume. The big difference in bubble volume could be partly

attributed to the thick pericarp which most hybrids possess.

Seed density is determined mainly by bubble volume for supersweet corn.

Seed conductivity is also affected by the shrinkage of the endosperm. The shrinkage of the endosperm is related to the accumulated sucrose, and consequently to the higher osmotic potential of the endosperm. Upon imbibition, the increase in the osmotic potential of the endosperm could hasten the water uptake in such a way that causes damage to the cell membrane and greater seed electrolyte leakage. A large bubble volume evidently can also cause an increase in seed electrolyte leakage.

Sweetness is directly influenced by the ability of hindering the conversion of sugar to the substrates for starch synthesis.

6.3.2 Correlations

The results showed that the four characters: pericarp thickness, bubble volume, seed density, and seed conductivity, were negatively correlated with all types of germination tests. All correlations were highly significant (Table 6.13). Highly significant, positive correlations also occurred among each of the four characters (Table 6.14). Seed weight and sweetness have been considered traditionally as two important factors which negatively affect germination rate, but this study reveals different

results.

Among the seven germination tests, the cold soil germination test had the lowest r^2 values with the six germination-related characters (Table 6.14). The low r^2 value could be caused by extremely high moisture content of the soil due to the rolled paper towel method or because of soil-borne fungi. In studies of Harper *et al.* (1955), seed mortality was greatest when soil moisture content was high. The cold soil test conducted in rolled paper towel tends to keep a higher moisture content than in germination pots.

Seed weight: Wann (1980) suggested that the nutrient reserve for the endosperm was critical and that increased seed weight should aid in germination. Andrew (1982) reported that among *sh2* inbred lines used as seed parents, seed weight was not related to germination rates or seedling vigor, but that within a seed parent, the largest seeds resulted in improved germination and seedling vigor. Bell *et al.* (1983) found that eleven cycles of mass selection in a population of *sh2* corn significantly increased field emergence and seed test weight. A highly significant correlation (r^2 = 74%**) was found between them.

This study shows that the correlations between seed weight and different germination rates varied. The coefficients of determination (r^2) are lower, ranging from 36.8% to 11.6%. The r^2 for seed weight and different conductivity data was 18.8%. The result from this study

suggests that the increase of seed weight does improve the seed germination ability, but the influence is not as strong as people usually consider.

In the case of Bell's (1983) *sh2* population, both the kernel test weight and field emergence were increased significantly, and there was a highly significant coefficient of determination (r^2 = 74%) between them, but it does not necessarily mean that the improvement of field emergence was mainly due to the increase of seed weight. The 11 cycles of mass selection of this *sh2* population were based on two criteria, seed weight and field emergence. Both were improved simultaneously, but the field emergence was influenced by many characters.

Pericarp thickness: It was traditionally thought that a thick pericarp might better resist internal pressure during kernel development and prevent splitting and exposure of the seed to pathogens (Helm and Zuber, 1969). This does not appear to be true for supersweet corn, in which increased germination rates arise from kernels possessing thinner pericarp over the range tested. It is probably that very thin pericarps (eg., under 40 μm) are much more easily fractured.

The hypothesis proposed here is that pericarp thickness affects germination of supersweet corn via its influence on the formation of bubble volume. Bubble volume is determined by both pericarp thickness and shrinkage of endosperm for *bt*

and *sh2* supersweet corn. Supersweet corn seeds that accumulate much less starch have a very high shrinkage of endosperm during kernel dry down. For these kernels, the pericarp thickness is a key factor in determining the bubble volume. An increase in stiffness should accompany a thick pericarp relative to a thin pericarp. During endosperm shrinkage, kernels possessing thick pericarps tend to experience greater separation of the endosperm from the pericarp than those having thin pericarps. In general, the kernel with thin pericarp tends to have a small bubble volume, whether the shrinkage of endosperm is substantial or not. Only kernels with thick pericarp and higher shrinkage of endosperm tend to have large bubble volume.

The r^2 of simple linear correlation of bubble volume with pericarp thickness and seed weight were 49.8% and 40.7%, respectively, and both were significant at the 1% level (Table 6.14). When pericarps were as thin as 60 μm, bubble volume became negligible, even with high shrinkage of endosperm (e.g. Hi38*bt* X HS*bt*). The pericarp thickness, therefore, is not expected to decrease continuously with breeding selections for high field emergence.

It is well-known that very thin pericarps (< 40 μm) are easily cracked, invaded by soil-borne pathogens. However, pericarp breakage related to large bubble volume is an important reason for poor germination rates. The disease resistance of variety per se is a key factor.

Tracy and Juvik (1989) reported that 11 cycles of mass selection for improved field emergence and kernel weight on a *sh2* population did not alter pericarp thickness. Field emergence and pericarp thickness were not correlated (r^2 = 6%). All kernels came from the same population with similar genetic background in their study. The differences among kernel pericarp thickness of different cycles (62 - 82 μm) was significant, but the differences may not have been large enough to cause the difference of bubble volume and influence the germination rate. On the other hand, the population was improved by selecting the 20% of the heaviest seeds (Bell *et al.*, 1983). The bubble volume itself and the differences among different seeds could be reduced by this process, since the heavier the seed weight, the smaller will be the shrinkage of endosperm, and it could also reduce the differences in seed weight. Tracy and Juvik's (1989) results should not contradict the present research. Pericarp thickness varied greatly (61 - 160 μm) and differences were highly significant in this study.

Pericarp thickness is one of the most important factors to determine the eating quality of supersweet corn besides its influence on germination. Bubble volume can be used as an index of visual selection for thin pericarp by supersweet corn breeders.

Bubble volume: Bubble volume affects seed viability negatively through several mechanisms: 1) the bubble volume

makes the pericarp more vulnerable to mechanical damage; 2) the bubble volume and the cracked pericarp create an ideal habitat for seed-borne and soil pathogens (Styer *et al.*, 1984); 3) the cracked pericarp facilitates water and electrolyte movement into and out of the seed (Wann, 1986; Douglass, *et al.*, 1993).

Bubble volume can also cause severe imbibition damage even with an intact pericarp, due to high electrolyte leakage. At imbibition, water and solutes pass through the hilar layer at the orifice for the kernels with intact pericarp. Rapid water movement into the seed increases the potential for damage or leakage during the initial phase of imbibition. For those seeds with no or little bubble volume, the loss of solutes could be hindered largely by the connection of endosperm and pericarp during the initial phase of imbibition. For seeds with an intact pericarp and bubble volume, however, the speed of water movement could be much faster through bubble volume than through the cross- and tube-cell zones of the pericarp, since there could be less resistance for water movement, and solutes could move much easier.

The hilar orifice, which is covered by the hilar layer, plays a very important role in the process of water uptake and solute leakage. In the process of measuring bubble volume, within a very short time (about 2 to 3 minutes), water completely filled the bubble volume by the force of

evacuation. One or two strings of mini-air bubbles come from each seed's basal end of the tip cap to the water surface. This phenomenon suggests that there should be a hilar orifice at the base of the seed, and the hilar layer that causes an early closure of the hilar orifice (Helen, 1935) may be broken through by the force of evacuation.

Wolf *et al.* (1952) found that for dent corn relatively little water was absorbed into the pericarp through the epidermal cells. Most of the water entering the kernel was taken up through the basal end of the tip cap, moving rapidly through the labyrinth of air spaces in the spongy parenchyma of the tip cap and the cross- and tube-cell zone of the pericarp. They also observed a hilar layer at the base of kernel and found that the pericarp showed semi-permeable characteristics with respect to solutes. Many inorganic substances failed to penetrate the pericarp. They suggested that the hilar layer may be more permeable to salts then the seed coat over the other parts of the seed.

Seed density: The difference of seed density of supersweet corn could be mainly influenced by bubble volume. It was found that the correlation between bubble volume and seed density was highly significant (r^2= 81%) (Table 6.2.2). Its relationships with pericarp thickness, conductivity, and germination rates were similar to those of the bubble volume's (Table 6.2.2). Seed density, therefore, could also be mainly influenced by both pericarp thickness and the

shrinkage of endosperm of different varieties of *bt* and *sh2* supersweet corn.

Seed conductivity: The seed leachate electrolyte conductivity was negatively correlated with germination tests and positively correlated with pericarp thickness, bubble volume, and seed density. All of these correlations were highly significant. The negatively and highly significant correlations between seed conductivity and the germination rates for both lab cold test and field emergence had been reported by many researchers. The results obtained here confirmed their conclusions.

Seed conductivity is a good indicator of seed germination ability.

Sweetness: It is traditionally considered that there is a negative correlation between kernel sweetness and germination rate. Douglass *et al.* (1993) reported that there was a highly significant negative correlation (r^2 = 55%) between the kernel sugar content and field emergence of sweet corn (*su*, *se* and *sh2*) field emergence in cold soil. This study provided different results from theirs. The correlations between sweetness and supersweet corn (*bt* and *sh2*) germination rates were not significant, with r^2 ranging from 0.6% to 9.9% only. The correlation between sweetness and conductivity was also very low, the value of with r^2 of 6.1%.

The reason that the other investigators obtained such

high r^2 values could be attributed to the *su* endosperm mutants. In Douglass *et al.* (1993) study, seven of 24 sweet corn genotypes were *su* and the rest were *se* and *sh2*. The genotypes with *su* gene had a much lower sucrose content mean (25 mg/g dry wt. vs. 35.5 of *se* and 41.9 of *sh2*) and much higher field emergence (69% vs. 46% of *se* and 44% of *sh2*).

The correlation between seed weight and sweetness was highly significant (r^2 = 70%) in this study. The sweeter the genotype is, the lower the seed weight.

Sweetness is one of the most important eating qualities of supersweet corn. The results from this study indicate that the interaction between endosperm gene and genetic background played an important role, and that germination ability of supersweet corn can be improved to a certain extent without decreasing sweetness.

CHAPTER 7

CONCLUSIONS

Viability: Viability and vigor were studied for corn endosperm mutants *su, bt* and *sh2* on a near-isogenic background. The *sh2* phenotype usually had the worst viability. The + phenotype performed best in different vigor tests, followed in turn by *su, bt* and *sh2*.

Accelerated aging (AA): Seed with poor quality suffered more than the seed of good quality when passed through AA. The net responses of *sh2* hybrids to AA in both germination and conductivity tests were larger than those of *bt* hybrids, followed in turn by *su* and + hybrids. The net responses of germination and conductivity to AA were correlated and significant at 1% level for the AA without drying and at 5% level for the AA after drying. It is evident that the electrolyte leakage caused by AA is highly correlated with the deterioration caused by AA. The accelerated aging, therefore, should be useful for predicting seed storability. Seeds with poor germination ability suffer more from AA, i.e., seeds with good germination ability may have better storability.

Pericarp thickness: These studies confirmed that pericarp thickness can be influenced greatly by endosperm mutant genes, probably through effects on inner pressure at different stage of kernel development. The influence of

inner pressure on pericarp was evident through investigation of pericarp thicknesses of isogenic hybrids at different days after pollination (DAP). Pericarps of wild type hybrids at 18 DAP were significantly thicker than the other three genotypes. However, at 36 DAP the *sh2* hybrids had highly significantly thicker pericarps than *bt, su* and wild type hybrids. On other hand, pericarp thicknesses of + and *sh2* hybrids at 36 DAP were significantly different from those at 18 DAP. A thinning trend of pericarp thickness was observed for the + hybrids from 18 to 36 DAP, while the pericarp thickness of the sh2 hybrids increased. There were no significant difference between 18 and 36 DAP for both *bt* and *su* hybrids.

These changes of pericarp thickness could be attributed to the interaction between inner pressure and increase of pericarp quantity. The highly significant r^2 values between seed weight and the difference of germinal, abgerminal, or average of pericarp thickness harvested at 18 and 36 DAP were also strong proofs for this inner pressure theory.

Sensory evaluation: The *su* mutant had the worst eating quality and *sh2* had the best for the three characters -- flavor, sweetness and tenderness -- although the difference between *bt* and *sh2* was not significant in many cases. Three *bt* hybrids with only Hawaii bred lines had much better eating qualities than the other *bt* and *sh2* hybrids whether cooked or not. The extensive genotypic variability for

these eating qualities among the *bt* hybrids suggests that the allelic variation at loci other than *bt* is probably involved. Pericarp thickness appears to affect tenderness more for kernels with higher sweetness than kernels with lower sweetness.

Since the sweetness of the three Hawaii bred *bt* hybrids have reached the level of commercial use, it can be safely concluded that the eating quality of the *bt*-type supersweet corn can compete with *sh2* type supersweet corn.

Germination-related characters: Six characters were studied in relation to germination -- seed weight, pericarp thickness, bubble volume (cavity between pericarp and endosperm), seed density, seed conductivity and sweetness. The six characters were influenced greatly by *bt* and *sh2* genes, and the differences caused by these two genes were highly significant. These differences could be largely attributed to the efficiency of *bt* and *sh2* genes in hindering conversion of sucrose to substrates for starch synthesis. The extensive genotypic variability for the six characters among the *bt* hybrids suggested that allelic variation at loci other than *bt* is involved.

Although it is agreed that the large bubble volume could influence seed viability adversely, the bubble volume has not been accurately quantified (Styer *et al.*, 1983: Wenn, 1986; Chern *et al.*, 1991; Douglass *et al.*, 1993).

A method of measuring bubble volume was developed,

based on the change of soapy water volume which replaced air in the bubble. The hypotheses were then tested that bubble volume was determined by pericarp thickness and shrinkage of endosperm, and thick pericarp lowers the germination rates of supersweet corn through its effect on the formation of large bubbles. Bubble volume can cause severe imbibition damage even with intact pericarps.

The correlations of germination with bubble volume, seed density, pericarp thickness, and seed conductivity were negative and highly significant. The correlation between seed weight and germination was positive and significant at a 5% level. Correlation between germination and sweetness was not significant. The correlations of germination with pericarp thickness, bubble volume, seed density and conductivity were highly significant, as were the correlations among these four characters. Since bubble volume is highly correlated with thick pericarp, it can be a very useful index of visual selection for breeders to improve tenderness of supersweet corn.

Appendix 1.

The data (%) of standard vs. cold germination tests of the 36 varieties.

		Standard test					Cold test				
Plot	Entry	Rep1	Rep2	Rep3	Avg	Avg	Rep1	Rep2	Rep3	Avg	Avg
1	Hi27+ X Hi38+	100	96	100	98.7		98	100	96	98.0	
2	Hi27+ X B37+	92	96	100	96.0		100	100	100	100.0	
3	Hi27+ X Oh43+	94	96	92	94.0		100	100	100	100.0	
4	Hi27+ X HS+	94	92	96	94.0		98	100	92	96.7	
14	Hi38+ X B37+	98	96	100	98.0		100	100	96	98.7	
15	Hi38+ X Oh43+	100	100	96	98.7		98	100	100	99.3	
16	Hi38+ X HS+	100	100	96	98.7		100	96	100	98.7	
24	Oh43+ X B37+	100	92	100	97.3		94	92	100	95.3	
28	HS+ X B37+	98	96	96	96.7	+	98	96	92	95.3	+
29	HS+ X Oh43+	92	100	100	97.3	96.9	98	100	100	99.3	98.1
17	Hi38bt X Hi27bt	72	60	76	69.3		78	72	72	74.0	
5	Hi27bt X B37bt	64	56	52	57.3		66	60	32	52.7	
6	Hi27bt X Oh43bt	58	68	52	59.3		48	64	32	48.0	
30	HSbt X Hi27bt	78	64	80	74.0		56	68	40	54.7	
18	Hi38bt X B37bt	84	80	96	86.7		68	72	44	61.3	
19	Hi38bt X Oh43bt	74	80	88	80.7		66	56	76	66.0	
20	Hi38bt X HSbt	78	88	84	83.3		74	80	84	79.3	
25	Oh43bt X B37bt	82	88	72	80.7		44	56	24	41.3	
31	HSbt X B37bt	86	80	72	79.3	*bt*	78	88	76	80.7	*bt*
32	HSbt X Oh43bt	92	88	100	93.3	76.4	88	84	88	86.7	64.5
7	Hi27sh2 X B37sh2	28	44	8	26.7		36	28	8	24.0	
8	Hi27sh2 X Oh43sh2	58	40	56	51.3		24	24	24	24.0	
9	Hi27sh2 X HSsh2	78	76	64	72.7		60	44	28	44.0	
26	Oh43sh2 X B37sh2	36	20	20	25.3		32	12	16	20.0	
33	HSsh2 X B37sh2	68	40	56	54.7	*sh2*	34	28	24	28.7	*sh2*
34	HSsh2 X Oh43sh2	76	80	80	78.7	51.6	72	52	48	57.3	33.0
10	Hi27su X Hi38su	92	92	96	93.3		98	80	80	86.0	
11	Hi27su X B37su	84	88	76	82.7		82	96	84	87.3	
12	Hi27su X Oh43su	82	80	52	71.3		72	96	52	73.3	
13	Hi27su X HSsu	96	96	96	96.0		94	92	84	90.0	
21	Hi38su X B37su	98	90	100	96.0		90	100	96	95.3	
22	Hi38su X Oh43su	78	80	84	80.7		96	92	92	93.3	
23	Hi38su X HSsu	96	100	100	98.7		94	92	100	95.3	
27	Oh43su X B37su	84	72	72	76.0		76	80	72	76.0	
35	HSsu X B37su	98	88	100	95.3	*su*	100	88	88	92.0	*su*
36	HSsu X Oh43su	94	80	92	88.7	87.9	88	84	92	88.0	87.7
	@MAX - @MIN	72.0	80.0	92.0	73.3		76.0	88.0	92.0	80.0	
	Average	82.8	80.1	80.6	81.1		77.7	77.0	70.3	75.0	
	CV%	20.9%	24.6%	28.6%	9.8%		29.5%	32.5%	42.9%	11.5%	

Appendix 2.

The data of conductivity (mIIli Siemens/meter) of the 36 varieties under 25 C temperature.

Plot	Entry	Rep1	Rep2	Rep3	Avg	Avg
1	Hi27+ X Hi38+	9.2	5.9	8.1	7.7	
2	Hi27+ X B37+	11.2	10.7	9.9	10.6	
3	Hi27+ X Oh43+	17.1	15.3	15.6	16.0	
4	Hi27+ X HS+	7.5	8.5	8.0	8.0	
14	Hi38+ X B37+	16.5	13.3	12.4	14.0	
15	Hi38+ X Oh43+	12.6	13.9	12.3	12.9	
16	Hi38+ X HS+	7.5	8.6	7.7	8.0	
24	Oh43+ X B37+	20.7	21.3	20.9	21.0	
28	HS+ X B37+	14.3	11.5	12.0	12.6	+
29	HS+ X Oh43+	15.2	11.5	15.2	14.0	12.5
17	Hi38bt X Hi27bt	13.3	8.9	9.3	10.5	
5	Hi27bt X B37bt	16.1	23.1	18.9	19.4	
6	Hi27bt X Oh43bt	27.3	22.0	27.4	25.6	
30	HSbt X Hi27bt	19.1	17.0	19.2	18.4	
18	Hi38bt X B37bt	15.5	16.4	15.2	15.7	
19	Hi38bt X Oh43bt	16.0	20.2	15.8	17.3	
20	Hi38bt X HSbt	11.7	10.2	14.0	12.0	
25	Oh43bt X B37bt	21.9	22.2	21.4	21.8	
31	HSbt X B37bt	13.7	17.7	20.3	17.2	*bt*
32	HSbt X Oh43bt	22.3	16.4	18.1	18.9	17.7
7	Hi27sh2 X B37sh2	31.3	29.7	27.6	29.5	
8	Hi27sh2 X Oh43sh2	28.6	33.0	27.9	29.8	
9	Hi27sh2 X HSsh2	16.8	18.5	19.3	18.2	
26	Oh43sh2 X B37sh2	39.4	30.7	28.3	32.8	
33	HSsh2 X B37sh2	27.3	28.9	32.4	29.5	*sh2*
34	HSsh2 X Oh43sh2	25.4	22.7	22.6	23.6	27.2
10	Hi27su X Hi38su	9.3	9.9	8.2	9.1	
11	Hi27su X B37su	17.8	16.2	17.4	17.1	
12	Hi27su X Oh43su	18.4	18.9	20.5	19.3	
13	Hi27su X HSsu	9.5	11.0	8.7	9.8	
21	Hi38su X B37su	22.2	13.7	15.2	17.0	
22	Hi38su X Oh43su	16.3	14.8	16.6	15.9	
23	Hi38su X HSsu	8.2	7.2	7.7	7.7	
27	Oh43su X B37su	22.8	24.7	22.4	23.3	
35	HSsu X B37su	13.7	9.9	12.8	12.1	*su*
36	HSsu X Oh43su	16.8	18.1	17.5	17.4	14.9
	@MAX - @MIN	31.9	27.2	24.7	25.1	
	Average	17.6	16.7	16.9	17.0	
	CV%	41.0%	41.9%	39.3%	12.6%	

Appendix 3.

The data (%) of AA (without drying) vs. AA (after drying) germination tests of the 36 varieties under 25 C temperature.

		AA without drying					AA after drying				
Plot	Entry	Rep1	Rep2	Rep3	Avg	Avg	Rep1	Rep2	Rep3	Avg	Avg
1	Hi27+ X Hi38+	96	88	96	93.3		100	88	100	96.0	
2	Hi27+ X B37+	96	96	96	96.0		100	92	92	94.7	
3	Hi27+ X Oh43+	100	96	100	98.7		100	84	100	94.7	
4	Hi27+ X HS+	100	96	96	97.3		100	96	100	98.7	
14	Hi38+ X B37+	96	100	92	96.0		92	96	96	94.7	
15	Hi38+ X Oh43+	100	100	100	100.0		100	96	96	97.3	
16	Hi38+ X HS+	100	100	100	100.0		100	100	100	100.0	
24	Oh43+ X B37+	88	100	96	94.7		92	88	92	90.7	
28	HS+ X B37+	100	92	100	97.3	+	96	96	100	97.3	+
29	HS+ X Oh43+	96	100	100	98.7	97.2	92	100	96	96.0	96.0
17	Hi38bt X Hi27bt	56	84	72	70.7		68	60	40	56.0	
5	Hi27bt X B37bt	48	32	52	44.0		32	25	20	25.7	
6	Hi27bt X Oh43bt	60	60	32	50.7		32	20	32	28.0	
30	HSbt X Hi27bt	60	68	64	64.0		48	32	28	36.0	
18	Hi38bt X B37bt	84	56	68	69.3		56	52	52	53.3	
19	Hi38bt X Oh43bt	72	56	72	66.7		32	44	30	35.3	
20	Hi38bt X HSbt	100	92	92	94.7		80	76	88	81.3	
25	Oh43bt X B37bt	56	72	60	62.7		32	36	60	42.7	
31	HSbt X B37bt	76	60	72	69.3	*bt*	60	32	68	53.3	*bt*
32	HSbt X Oh43bt	96	76	76	82.7	67.5	70	76	68	71.3	48.3
7	Hi27sh2 X B37sh2	32	24	24	26.7		12	16	12	13.3	
8	Hi27sh2 X Oh43sh2	28	32	44	34.7		32	4	4	13.3	
9	Hi27sh2 X HSsh2	44	48	60	50.7		28	16	28	24.0	
26	Oh43sh2 X B37sh2	8	16	16	13.3		4	4	12	6.7	
33	HSsh2 X B37sh2	28	28	32	29.3	*sh2*	8	4	12	8.0	*sh2*
34	HSsh2 X Oh43sh2	64	52	44	53.3	34.7	24	28	40	30.7	16.0
10	Hi27su X Hi38su	100	92	100	97.3		84	96	96	92.0	
11	Hi27su X B37su	88	84	88	86.7		68	72	72	70.7	
12	Hi27su X Oh43su	60	44	76	60.0		48	28	60	45.3	
13	Hi27su X HSsu	96	100	68	88.0		96	92	84	90.7	
21	Hi38su X B37su	100	76	92	89.3		76	88	76	80.0	
22	Hi38su X Oh43su	92	84	84	86.7		72	60	88	73.3	
23	Hi38su X HSsu	100	96	92	96.0		88	88	88	88.0	
27	Oh43su X B37su	56	68	72	65.3		64	28	44	45.3	
35	HSsu X B37su	96	88	88	90.7	*su*	80	92	76	82.7	*su*
36	HSsu X Oh43su	56	72	88	72.0	83.2	80	76	96	84.0	75.2
	@MAX - @MIN	92.0	84.0	84.0	86.7		96.0	96.0	96.0	93.3	
	Average	75.8	73.0	75.1	74.6		65.2	60.6	65.2	63.6	
	CV%	34.6%	34.6%	32.0%	11.9%		46.5%	55.1%	49.1%	16.5%	

Appendix 4.

The data (%) of cold soil germination test of the 36 varieties (sand : soil = 2:1).

Plot	Entry	Rep1	Rep2	Rep3	Avg	
1	Hi27+ X Hi38+	68	72	64	68.0	
2	Hi27+ X B37+	88	56	86	76.7	
3	Hi27+ X Oh43+	32	56	64	50.7	
4	Hi27+ X HS+	56	60	94	70.0	
14	Hi38+ X B37+	76	60	72	69.3	
15	Hi38+ X Oh43+	52	64	76	64.0	
16	Hi38+ X HS+	92	76	100	89.3	
24	Oh43+ X B37+	44	52	40	45.3	
28	HS+ X B37+	92	88	92	90.7	+
29	HS+ X Oh43+	80	84	92	85.3	70.9
17	Hi38bt X Hi27bt	60	40	32	44.0	
5	Hi27bt X B37bt	12	10	6	9.3	
6	Hi27bt X Oh43bt	12	8	6	8.7	
30	HSbt X Hi27bt	60	20	40	40.0	
18	Hi38bt X B37bt	40	38	16	31.3	
19	Hi38bt X Oh43bt	24	20	40	28.0	
20	Hi38bt X HSbt	28	20	32	26.7	
25	Oh43bt X B37bt	20	20	28	22.7	
31	HSbt X B37bt	48	24	56	42.7	*bt*
32	HSbt X Oh43bt	52	28	60	46.7	30.0
7	Hi27sh2 X B37sh2	8	10	10	9.3	
8	Hi27sh2 X Oh43sh2	16	8	8	10.7	
9	Hi27sh2 X HSsh2	36	56	28	40.0	
26	Oh43sh2 X B37sh2	12	0	8	6.7	
33	HSsh2 X B37sh2	20	10	10	13.3	*sh2*
34	HSsh2 X Oh43sh2	12	12	16	13.3	15.6
10	Hi27su X Hi38su	44	52	72	56.0	
11	Hi27su X B37su	36	44	56	45.3	
12	Hi27su X Oh43su	26	64	36	42.0	
13	Hi27su X HSsu	24	64	60	49.3	
21	Hi38su X B37su	84	60	62	68.7	
22	Hi38su X Oh43su	74	70	52	65.3	
23	Hi38su X HSsu	64	86	44	64.7	
27	Oh43su X B37su	20	32	26	26.0	
35	HSsu X B37su	80	50	80	70.0	*su*
36	HSsu X Oh43su	56	54	92	67.3	55.5
	@MAX - @MIN	84.0	88.0	94.0	84.0	
	Average	45.8	43.6	48.8	46.0	
	CV%	57.2%	58.4%	60.0%	20.4%	

Appendix 5.

The data of conductivity of AA (without drying) vs. AA (after drying) of the 36 varieties under 25 C temperature (milli Siemens/meter).

		AA (without drying)					AA (after dring)				
Plot	Entry	Rep1	Rep2	Rep3	Avg	Avg	Rep1	Rep2	Rep3	Avg	Avg
1	Hi27+ X Hi38+	8.6	10.4	10.0	9.7		10.0	7.4	9.9	9.1	
2	Hi27+ X B37+	12.1	13.8	9.9	12.0		11.6	11.0	11.7	11.4	
3	Hi27+ X Oh43+	17.9	18.7	20.1	18.9		16.3	16.4	16.6	16.4	
4	Hi27+ X HS+	7.5	10.2	9.6	9.1		8.2	10.0	7.2	8.4	
14	Hi38+ X B37+	14.2	15.5	16.1	15.3		13.8	13.6	13.6	13.7	
15	Hi38+ X Oh43+	14.6	13.5	14.5	14.2		15.3	15.0	11.9	14.1	
16	Hi38+ X HS+	9.1	8.5	8.0	8.6		9.4	8.1	9.8	9.1	
24	Oh43+ X B37+	21.3	25.3	20.7	22.4		21.5	24.1	22.4	22.7	
28	HS+ X B37+	13.9	17.6	14.5	15.3	+	13.0	14.6	12.7	13.4	+
29	HS+ X Oh43+	16.4	16.4	14.7	15.8	14.1	14.5	12.9	13.0	13.4	13.2
17	Hi38bt X Hi27bt	14.4	13.4	19.3	15.7		16.6	13.8	15.9	15.5	
5	Hi27bt X B37bt	20.1	27.1	26.1	24.4		19.2	19.0	20.9	19.7	
6	Hi27bt X Oh43bt	37.5	45.6	27.5	36.9		25.4	35.3	31.4	30.7	
30	HSbt X Hi27bt	26.7	24.9	21.8	24.5		28.8	22.5	29.5	26.9	
18	Hi38bt X B37bt	20.8	19.3	23.8	21.3		18.9	18.4	20.9	19.4	
19	Hi38bt X Oh43bt	21.6	29.3	22.2	24.4		21.5	16.4	15.7	17.9	
20	Hi38bt X HSbt	17.4	16.0	16.8	16.7		21.2	13.1	13.9	16.1	
25	Oh43bt X B37bt	25.5	28.7	28.9	27.7		28.1	27.3	27.4	27.6	
31	HSbt X B37bt	22.3	26.7	24.7	24.6	*bt*	21.1	23.3	23.9	22.8	*bt*
32	HSbt X Oh43bt	16.2	15.5	21.5	17.7	23.4	19.6	16.9	15.3	17.3	21.4
7	Hi27sh2 X B37sh2	45.1	39.9	41.8	42.3		34.5	41.8	41.3	39.2	
8	Hi27sh2 X Oh43sh2	40.7	44.2	39.2	41.4		37.6	24.8	27.9	30.1	
9	Hi27sh2 X HSsh2	26.7	23.6	25.2	25.2		24.0	24.8	25.4	24.7	
26	Oh43sh2 X B37sh2	34.5	42.2	39.7	38.8		40.2	36.4	32.5	36.4	
33	HSsh2 X B37sh2	41.1	37.5	45.4	41.3	*sh2*	32.2	33.6	36.5	34.1	*sh2*
34	HSsh2 X Oh43sh2	32.4	32.6	27.2	30.7	36.6	27.1	32.2	33.6	31.0	32.6
10	Hi27su X Hi38su	14.5	12.1	13.0	13.2		9.8	11.2	11.3	10.7	
11	Hi27su X B37su	17.2	20.0	19.1	18.8		18.7	22.3	18.4	19.8	
12	Hi27su X Oh43su	24.2	35.6	22.3	27.4		24.9	23.8	23.4	24.0	
13	Hi27su X HSsu	11.6	12.4	9.6	11.2		10.0	8.2	9.2	9.1	
21	Hi38su X B37su	14.9	13.0	13.7	13.9		13.7	14.3	13.1	13.7	
22	Hi38su X Oh43su	15.8	18.2	17.9	17.3		16.6	15.5	15.7	15.9	
23	Hi38su X HSsu	9.7	13.1	8.8	10.5		8.2	9.0	7.0	8.1	
27	Oh43su X B37su	27.0	27.7	24.7	26.5		26.6	25.6	31.5	27.9	
35	HSsu X B37su	14.4	12.2	14.4	13.7	*su*	13.4	14.6	13.8	13.9	*su*
36	HSsu X Oh43su	19.4	18.8	22.5	20.2	17.3	21.6	20.3	19.9	20.6	16.4
	@MAX - @MIN	37.6	37.1	37.4	33.7		32.02	34.45	34.34	31.2	
	Average	20.8	22.2	21.0	21.3		19.8	19.4	19.6	19.6	
	CV%	46.6%	47.2%	44.9%	12.6%		42.0%	45.0%	45.9%	12.2%	

Appendix 6.

Data of the pericarp thickness of the 19 parental inbred lines.

(Germinal side + Abgerminal side)/2; (microns)

Parental lines	1	2	3	4	5	6	7	8	9	10	Avg	Avg
Hi27+	84.5	90.5	91.0	81.0	96.5	103.5	107.5	79.0	79.0	78.5	89.1	
Hi27bt	93.5	79.5	81.0	104.0	74.0	87.0	77.5	110.5	76.5	75.0	85.9	
Hi27sh2	87.0	121.5	118.5	77.0	112.5	131.5	129.5	122.0	68.5	115.0	108.3	
Hi27su	57.0	54.0	55.0	78.5	83.0	75.5	75.0	62.0	84.0	77.0	70.1	88.3
HS+	91.5	88.5	87.5	79.0	83.5	64.5	67.5	80.0	100.0	72.5	81.5	
HSbt	99.5	67.5	45.0	72.0	66.5	73.5	50.5	69.0	96.0	43.5	68.3	
HSsh2	72.5	80.5	74.5	87.5	81.5	75.0	109.0	110.0	75.0	110.5	87.6	
HSsu	66.0	63.5	91.5	93.0	59.0	67.0	75.0	65.5	76.5	77.0	73.4	77.7
B37+	104.5	106.0	93.5	137.5	116.0	106.0	146.5	103.0	127.5	154.0	119.5	
B37bt	165.5	141.0	181.0	131.5	200.5	213.5	217.5	221.5	165.0	154.5	179.2	
B37sh2	186.5	170.0	185.5	155.0	164.5	201.0	185.0	210.0	159.0	205.0	182.2	
B37su	132.5	134.5	111.5	127.5	89.5	128.5	127.0	117.5	127.5	128.0	122.4	150.8
Oh43+	52.5	69.5	79.0	76.0	73.5	52.5	69.5	73.5	71.5	63.5	68.1	
Oh43bt	98.0	112.0	121.0	95.0	92.0	108.0	97.0	108.0	117.5	108.0	105.7	
Oh43sh2	139.5	127.5	111.0	140.5	119.0	120.5	136.0	112.5	183.0	104.0	129.4	
Oh43su	98.5	100.0	101.0	78.5	103.5	93.0	85.5	76.0	90.5	95.0	92.2	98.8
Hi38+	67.5	70.0	76.0	72.0	80.0	57.0	64.0	67.5	76.0	71.0	70.1	
Hi38bt	56.5	55.0	56.5	63.0	47.0	52.0	64.5	69.5	57.0	61.0	58.2	
Hi38su	69.0	63.0	71.0	67.0	58.0	55.0	71.0	63.5	56.5	71.5	64.6	

Appendix 7.

Data of the pericarp thickness of the 36 varieties at 18 days after pollination (DAP) (microns) (Germinal side + Abgerminal side)/2

Plot	Entry	1	2	3	4	5	6	7	8	9	10	Avg	Avg
	Without Hi38:												
2	Hi27+ X B37+	156.0	137.0	142.5	156.5	133.0	120.5	153.5	152.5	149.0	133.5	143.4	
3	Hi27+ X Oh43+	121.0	143.5	106.5	106.0	114.5	120.5	112.0	138.0	138.5	126.0	122.7	
4	Hi27+ X HS+	95.0	96.5	112.0	87.0	99.0	82.5	98.0	103.5	108.5	97.0	97.9	
24	Oh43+ X B37+	135.0	129.5	114.5	119.5	133.0	102.5	120.0	95.5	125.0	110.5	118.5	
28	HS+ X B37+	102.5	134.5	113.5	98.5	95.0	121.5	145.0	121.0	129.0	134.5	119.5	+
29	HS+ X Oh43+	117.5	108.0	107.5	127.5	124.5	116.5	117.0	109.0	119.5	124.0	117.1	119.8
5	Hi27bt X B37bt	116.5	95.0	102.0	95.0	96.5	104.0	113.5	101.0	99.0	118.0	104.1	
6	Hi27bt X Oh43bt	113.0	81.0	110.0	80.5	80.5	76.0	92.0	64.5	82.0	83.5	86.3	
30	HSbt X Hi27bt	86.0	76.0	59.0	65.0	59.5	65.5	54.0	69.5	72.5	66.5	67.4	
25	Oh43bt X B37bt	110.5	124.5	82.0	113.5	93.5	119.0	91.5	111.5	93.0	94.5	103.4	
31	HSbt X B37bt	93.0	89.5	90.5	73.0	79.5	76.5	76.0	69.0	66.5	71.5	78.5	*bt*
32	HSbt X Oh43bt	82.0	72.5	82.0	63.0	80.0	75.0	76.5	62.0	76.0	79.5	74.9	85.7
7	Hi27sh2 X B37sh2	88.0	103.5	91.0	86.5	90.5	74.5	93.5	90.5	92.5	86.0	89.7	
8	Hi27sh2 X Oh43sh2	70.0	78.5	99.5	101.5	79.0	85.0	81.5	76.0	80.5	90.0	84.2	
9	Hi27sh2 X HSsh2	86.5	75.5	78.5	135.5	89.0	97.0	91.5	87.5	96.0	100.5	93.8	
26	Oh43sh2 X B37sh2	97.0	114.5	98.5	86.0	81.5	103.5	97.5	71.0	92.0	90.0	93.2	
33	HSsh2 X B37sh2	107.5	91.0	97.0	89.0	92.0	80.0	86.0	83.0	89.5	97.0	91.2	*sh2*
34	HSsh2 X Oh43sh2	90.0	68.5	74.5	76.0	72.5	83.5	75.0	60.0	50.0	60.0	71.0	87.2
11	Hi27su X B37su	83.0	125.5	127.5	90.5	115.0	119.0	111.0	103.5	104.0	110.5	109.0	
12	Hi27su X Oh43su	94.0	106.0	120.5	91.0	85.0	77.5	93.0	104.0	97.0	93.0	96.1	
13	Hi27su X HSsu	79.5	90.0	89.5	83.5	84.5	86.5	83.5	80.5	77.5	92.0	84.7	
27	Oh43su X B37su	94.5	109.0	109.0	99.5	98.0	70.5	87.0	115.0	99.0	103.5	98.5	
35	HSsu X B37su	108.0	116.0	104.0	109.5	107.5	104.0	101.5	98.0	118.0	115.5	108.2	*su*
36	HSsu X Oh43su	87.5	71.5	75.5	101.5	69.5	69.0	81.5	74.0	72.5	63.5	76.6	95.5
	With HI38												
1	Hi27+ X Hi38+	102.5	112.5	106.0	103.0	94.5	107.0	113.0	101.0	98.0	104.5	104.2	
14	Hi38+ X B37+	113.0	120.0	116.5	132.5	123.5	124.0	105.0	111.5	111.5	116.0	117.4	
15	Hi38+ X Oh43+	96.5	100.0	98.0	94.5	101.0	118.5	107.0	90.5	93.5	92.5	99.2	+
16	Hi38+ X HS+	64.0	67.5	97.5	65.5	103.5	110.0	72.0	74.0	103.5	96.5	85.4	101.5
17	Hi38bt X Hi27bt	67.0	64.0	74.0	62.5	69.0	75.5	99.0	76.0	79.5	61.5	72.8	
18	Hi38bt X B37bt	88.0	83.5	77.0	97.0	82.0	102.5	86.0	81.5	84.0	89.5	87.1	
19	Hi38bt X Oh43bt	77.5	72.5	82.5	105.5	64.5	78.0	82.5	97.0	87.5	69.5	81.7	*bt*
20	Hi38bt X HSbt	43.0	54.5	42.5	54.0	44.5	48.5	39.5	46.5	44.0	52.0	46.9	72.1
10	Hi27su X Hi38su	90.5	84.5	74.5	94.0	100.0	86.0	90.5	86.0	83.0	96.0	88.5	
21	Hi38su X B37su	126.0	124.5	91.5	106.5	101.0	92.0	101.0	119.5	100.5	100.0	106.3	
22	Hi38su X Oh43su	81.5	73.0	83.5	88.5	68.0	78.5	67.5	70.5	76.5	65.5	75.3	*su*
23	Hi38su X HSsu	71.5	70.5	80.5	56.5	63.0	77.5	55.5	72.0	57.5	68.5	67.3	84.3
	Average											93.4	
	CV%											11.7%	

Appendix 8.

Data of the pericarp thickness of the 36 varieties at 36 days after pollination (DAP) (microns) (Germinal side + Abgerminal side)/2.

Plot	Entry	1	2	3	4	5	6	7	8	9	10	Avg	Avg
Without Hi38:													
2	Hi27+ X B37+	139.0	110.0	102.5	93.0	118.5	105.0	137.0	112.0	107.0	109.0	113.3	
3	Hi27+ X Oh43+	93.5	94.0	94.0	90.0	83.0	99.5	79.0	96.5	83.5	106.5	92.0	
4	Hi27+ X HS+	65.0	76.0	70.5	94.5	76.0	81.5	70.0	91.5	71.0	71.0	76.7	
24	Oh43+ X B37+	118.5	87.5	102.5	111.5	101.5	83.5	88.5	91.0	83.5	110.0	97.8	
28	HS+ X B37+	86.0	77.0	84.5	84.0	86.0	94.5	78.0	78.0	94.5	94.0	85.7	+
29	HS+ X Oh43+	63.5	84.5	94.5	105.5	67.0	89.5	74.0	76.5	63.0	64.0	78.2	90.6
5	Hi27bt X B37bt	141.0	107.5	113.5	134.0	141.5	126.5	130.0	117.0	116.0	153.0	128.0	
6	Hi27bt X Oh43bt	100.5	116.5	106.5	114.0	95.0	109.5	95.0	111.5	124.5	117.0	109.0	
30	HSbt X Hi27bt	106.5	108.0	97.5	85.0	101.5	85.5	109.0	100.5	105.0	98.0	99.7	
25	Oh43bt X B37bt	104.5	120.0	125.5	105.0	144.0	137.5	112.0	139.0	109.5	121.5	121.9	
31	HSbt X B37bt	115.0	137.5	99.0	90.5	105.0	106.0	109.5	114.5	96.5	106.0	108.0	*bt*
32	HSbt X Oh43bt	76.0	63.5	71.5	66.0	60.0	68.5	70.5	69.0	79.5	68.5	69.3	106.0
7	Hi27sh2 X B37sh2	160.0	170.5	160.5	165.0	177.0	155.5	167.0	173.5	191.0	77.5	159.8	
8	Hi27sh2 X Oh43sh2	142.5	162.0	139.0	136.5	159.0	142.5	140.0	132.0	154.5	109.0	141.7	
9	Hi27sh2 X HSsh2	114.0	95.5	99.5	107.5	101.0	114.0	114.0	129.5	128.5	106.5	111.0	
26	Oh43sh2 X B37sh2	140.5	153.5	125.0	116.5	133.0	137.5	162.0	171.0	121.5	112.0	137.3	
33	HSsh2 X B37sh2	183.0	141.5	151.5	171.5	141.0	157.0	157.0	189.5	155.5	142.0	159.0	*sh2*
34	HSsh2 X Oh43sh2	151.5	115.5	88.5	153.5	142.0	144.5	98.0	141.0	132.0	127.5	129.4	139.7
11	Hi27su X B37su	112.5	105.0	118.5	123.5	111.0	116.5	117.0	119.5	117.5	153.0	119.4	
12	Hi27su X Oh43su	81.0	96.5	95.5	87.5	64.5	79.5	88.5	96.5	95.5	117.0	90.2	
13	Hi27su X HSsu	57.0	64.5	82.0	73.0	54.5	50.0	85.0	77.5	67.0	59.5	67.0	
27	Oh43su X B37su	105.5	99.0	118.0	101.5	92.0	100.5	98.5	98.5	103.0	100.0	101.7	
35	HSsu X B37su	110.0	119.0	132.0	91.5	90.0	80.0	107.0	118.0	96.0	92.5	103.6	*su*
36	HSsu X Oh43su	110.0	100.5	87.0	112.5	94.5	97.5	101.0	93.0	76.5	100.5	97.3	96.5
	With Hi38												
1	Hi27+ X Hi38+	80.0	91.0	67.5	77.5	90.5	87.0	83.5	72.0	89.0	77.5	81.6	
14	Hi38+ X B37+	84.0	81.0	88.5	84.0	85.0	96.5	101.5	75.5	95.0	85.5	87.7	
15	Hi38+ X Oh43+	81.5	89.0	73.5	85.0	80.5	93.5	82.0	80.0	82.5	87.5	83.5	+
16	Hi38+ X HS+	101.0	75.5	63.5	69.5	102.5	94.0	61.0	85.0	61.0	87.0	80.0	83.2
17	Hi38bt X Hi27bt	93.5	78.0	63.0	88.5	95.5	74.0	81.5	85.5	84.5	75.0	81.9	
18	Hi38bt X B37bt	78.0	109.0	106.0	97.0	79.5	98.0	107.0	110.0	110.0	88.0	98.3	
19	Hi38bt X Oh43bt	89.0	77.5	83.0	85.5	84.5	82.5	82.0	86.5	79.0	79.0	82.9	*bt*
20	Hi38bt X HSbt	60.0	50.0	69.5	64.0	55.0	61.0	69.5	73.5	50.5	60.5	61.4	81.1
10	Hi27su X Hi38su	78.5	72.5	74.5	70.0	67.0	95.5	66.0	62.0	65.5	71.0	72.3	
21	Hi38su X B37su	88.5	100.5	93.0	116.0	117.0	118.5	91.0	100.5	98.5	123.0	104.7	
22	Hi38su X Oh43su	102.5	87.5	98.0	85.5	108.5	81.5	86.0	98.5	100.0	81.0	92.9	*su*
23	Hi38su X HSsu	81.5	91.0	75.5	58.5	59.5	60.0	55.0	87.5	49.5	62.0	68.0	84.5
	Average											99.8	
	CV%											13.3%	

Appendix 9.

Data of the germinal, abgerminal and average pericarp thickness of the 36 varietis harvested at 18 and 36 days after pollination (DAP), and the differences (microns).

Plot	Entry	18DAP Germ	36DAP Germ	36-18 Diff.	18DAP Abg.	36DAP Abg	36-18 Diff.	18DAP Avg	36DAP Avg	36-18 Diff.	Seed weight
2	Hi27+ X B37+	139.5	93.7	-45.8	147.3	132.9	-14.4	143.4	113.3	-30.1	26.2
3	Hi27+ X Oh43+	117.2	78.6	-38.6	128.1	105.3	-22.8	122.7	92.0	-30.7	25.9
4	Hi27+ X HS+	88.2	64.9	-23.3	107.6	88.5	-19.1	97.9	76.7	-21.2	26.3
24	Oh43+ X B37+	107.3	81.2	-26.1	129.7	114.4	-15.3	118.5	97.8	-20.7	25.1
28	HS+ X B37+	109.5	74.9	-34.6	129.5	96.4	-33.1	119.5	85.7	-33.9	28.1
29	HS+ X Oh43+	92.7	66.1	-26.6	141.5	90.3	-51.2	117.1	78.2	-38.9	25.7
1	Hi27+ X Hi38+	97.3	68.9	-28.4	111.1	94.2	-16.9	104.2	81.6	-22.7	27.4
14	Hi38+ X B37+	101.0	78.6	-22.4	133.7	96.7	-37.0	117.4	87.7	-29.7	27.1
15	Hi38+ X Oh43+	88.8	73.3	-15.5	109.6	93.7	-15.9	99.2	83.5	-15.7	27.5
16	Hi38+ X HS+	81.0	69.6	-11.4	89.8	90.4	0.6	85.4	80.0	-5.4	22.7
5	Hi27bt X B37bt	102.2	105.7	3.5	105.9	150.3	44.4	104.1	128.0	24.0	17.1
6	Hi27bt X Oh43bt	78.1	85.1	7.0	94.5	132.9	38.4	86.3	109.0	22.7	18.4
25	Oh43bt X B37bt	98.6	90.9	-7.7	108.1	152.8	44.7	103.4	121.9	18.5	18.8
30	HSbt X Hi27bt	67.0	80.0	13.0	67.7	119.3	51.6	67.4	99.7	32.3	12.3
31	HSbt X B37bt	77.6	87.7	10.1	79.4	128.2	48.8	78.5	108.0	29.5	18.4
32	HSbt X Oh43bt	72.5	51.5	-21.0	77.2	87.1	9.9	74.9	69.3	-5.5	19.6
17	Hi38bt X Hi27bt	66.7	71.4	4.7	78.9	92.4	13.5	72.8	81.9	9.1	12.8
18	Hi38bt X B37bt	76.6	82.2	5.6	97.6	114.3	16.7	87.1	98.3	11.2	16.6
19	Hi38bt X Oh43bt	75.4	74.3	-1.1	88.0	91.4	3.4	81.7	82.9	1.1	16.8
20	Hi38bt X HSbt	51.3	55.8	4.5	42.5	66.9	24.4	46.9	61.4	14.5	13.3
7	Hi27sh2 X B37sh2	88.0	138.0	50.0	91.3	181.5	90.2	89.7	159.8	70.1	11.1
8	Hi27sh2 X Oh43sh2	79.7	114.3	34.6	88.6	169.1	80.5	84.2	141.7	57.5	11.5
9	Hi27sh2 X HSsh2	94.4	87.5	-6.9	93.1	134.5	41.4	93.8	111.0	17.3	13.0
26	Oh43sh2 X B37sh2	92.7	112.1	19.4	93.6	162.4	68.8	93.2	137.3	44.1	10.8
33	HSsh2 X B37sh2	88.1	121.6	33.5	94.3	196.3	102.0	91.2	159.0	67.7	12.4
34	HSsh2 X Oh43sh2	67.9	96.5	28.6	74.1	162.3	88.2	71.0	129.4	58.4	13.3
11	Hi27su X B37su	103.0	101.0	-2.0	114.9	137.8	22.9	109.0	119.4	10.5	21.7
12	Hi27su X Oh43su	95.5	76.2	-19.3	96.7	104.2	7.5	96.1	90.2	-5.9	20.7
13	Hi27su X HSsu	80.3	56.2	-24.1	89.1	77.8	-11.3	84.7	67.0	-17.7	19.9
27	Oh43su X B37su	103.7	80.5	-23.2	93.3	122.8	29.5	98.5	101.7	3.2	19.5
35	HSsu X B37su	96.3	81.3	-15.0	120.1	125.9	5.8	108.2	103.6	-4.6	23.0
36	HSsu X Oh43su	74.0	81.3	7.3	79.2	113.3	34.1	76.6	97.3	20.7	20.6
10	Hi27su X Hi38su	90.9	65.5	-25.4	86.1	79.0	-7.1	88.5	72.3	-16.3	21.0
21	Hi38su X B37su	103.2	83.0	-20.2	109.3	126.3	17.0	106.3	104.7	-1.6	24.2
22	Hi38su X Oh43su	80.3	77.2	-3.1	70.3	108.6	38.3	75.3	92.9	17.6	21.7
23	Hi38su X HSsu	64.6	52.8	-11.8	70.0	83.2	13.2	67.3	68.0	0.7	22.0

Correlations: Differences of PT (36DAP - 18DAP) responses with seed weight.

		R Squared
Difference of germinal side (36-18) vs. seed weight	(-)	70.4% **
Difference of abgerminal side (36-18) vs. seed weight	(-)	70.0% **
Difference of average PT (36-18) vs. seed weight	(-)	74.0% **

a. *, ** Significant differences at the 5% and 1% levels, respectively.

Appendix 10.

Data of the bubble volume (ml/100 seeds).

(bt1 and sh2 only)

Plot	Entry	Sample1		Sample2		Sample3		Bubble volume			
		V1 *	V2	V1	V2	V1	V2	Sam1	Sam2	Sam3	Avg
5	Hi27bt X B37bt	65.0	63.2	68.0	66.0	66.0	63.8	1.80	2.00	2.20	2.00
6	Hi27bt X Oh43bt	77.0	75.6	75.3	74.0	66.4	65.0	1.40	1.30	1.40	1.37
17	Hi38bt X Hi27bt	64.0	62.4	72.2	71.1	62.8	61.0	1.60	1.10	1.80	1.50
18	Hi38bt X B37bt	65.0	64.5	63.9	63.0	64.3	63.8	0.50	0.90	0.50	0.63
19	Hi38bt X Oh43bt	64.5	63.4	64.9	64.0	66.5	66.0	1.10	0.90	0.50	0.83
20	Hi38bt X HSbt	61.1	61.0	60.9	60.2	60.5	60.3	0.10	0.70	0.20	0.33
25	Oh43bt X B37bt	65.8	65.0	63.9	63.6	67.9	67.0	0.80	0.30	0.90	0.67
30	HSbt X Hi27bt	62.8	61.0	63.0	61.0	63.5	62.0	1.80	2.00	1.50	1.77
31	HSbt X B37bt	67.2	66.1	66.1	65.0	68.2	67.0	1.10	1.10	1.20	1.13
32	HSbt X Oh43bt	68.0	67.0	67.0	66.0	67.4	66.3	1.00	1.00	1.10	1.03
7	Hi27sh2 X B37sh2	73.0	69.6	75.0	72.6	66.0	62.9	3.40	2.40	3.10	2.97
8	Hi27sh2 X Oh43sh2	64.0	61.0	65.0	63.0	62.3	60.5	3.00	2.00	1.80	2.27
9	Hi27sh2 X HSsh2	64.0	62.0	63.0	61.3	64.1	62.3	2.00	1.70	1.80	1.83
26	Oh43sh2 X B37sh2	63.9	60.5	65.0	61.0	65.0	61.0	3.40	4.00	4.00	3.80
33	HSsh2 X B37sh2	63.9	61.2	63.8	61.9	62.0	60.4	2.70	1.90	1.60	2.07
34	HSsh2 X Oh43sh2	66.9	63.1	67.6	63.9	66.0	63.0	3.80	3.70	3.00	3.50
	@MAX - @MIN							3.70	3.70	3.80	3.73
	Average							1.84	1.69	1.66	1.73
	CV%							0.61	0.61	0.62	20.1%

* V1 is the volume of 100 seeds immersed in a certain amount soapy warer.
V2 is the volume of the same sample but after vaccuming for about
2-3 minutes which forced out almost all of the air in the bubble space.
Bubble volume = V1 - V2.

Appendix 11.

Data of the seed density of the 16 varieties.
(bt1 and sh2 only) (Gram/ml)

Plot	Entry	Samp1	Samp2	Samp3	Avg	Avg
5	Hi27bt X B37bt	0.99	0.98	0.98	0.98	
6	Hi27bt X Oh43bt	1.10	1.05	1.11	1.09	
17	Hi38bt X Hi27bt	1.06	1.12	1.02	1.07	
18	Hi38bt X B37bt	1.25	1.12	1.16	1.18	
19	Hi38bt X Oh43bt	1.16	1.14	1.18	1.16	
20	Hi38bt X HSbt	1.22	1.18	1.21	1.20	
25	Oh43bt X B37bt	1.14	1.23	1.61	1.33	
30	HSbt X Hi27bt	1.02	0.97	1.12	1.04	
31	HSbt X B37bt	1.15	1.15	1.20	1.16	*bt*
32	HSbt X Oh43bt	1.11	1.15	1.11	1.12	1.13
7	Hi27sh2 X B37sh2	0.85	0.90	0.85	0.87	
8	Hi27sh2 X Oh43sh2	0.85	0.86	0.94	0.88	
9	Hi27sh2 X HSsh2	0.99	1.00	0.95	0.98	
26	Oh43sh2 X B37sh2	0.81	0.86	0.87	0.85	
33	HSsh2 X B37sh2	0.86	0.94	0.97	0.92	*sh2*
34	HSsh2 X Oh43sh2	0.89	0.92	0.93	0.92	0.90
	@MAX - @MIN	0.45	0.37	0.76	0.48	
	Average	1.03	1.04	1.08	**1.05**	
	CV%	0.14	0.12	0.17	7.1%	

Appendix 12.

Data of the seed weight of the 36 varietis.
(gram/100 seeds)

Plot	Entry	Samp1	Samp2	Samp3	Avg	Avg
1	Hi27+ X Hi38+	28.56	26.25	27.43	27.4	
2	Hi27+ X B37+	26.6	26.21	25.79	26.2	
3	Hi27+ X Oh43+	25.81	25.49	26.47	25.9	
4	Hi27+ X HS+	26.3	26.2	26.46	26.3	
14	Hi38+ X B37+	27.32	27.59	26.39	27.1	
15	Hi38+ X Oh43+	27.85	27.15	27.37	27.5	
16	Hi38+ X HS+	23.46	23.24	21.41	22.7	
24	Oh43+ X B37+	24.83	24.86	25.63	25.1	
28	HS+ X B37+	27.92	27.86	28.63	28.1	+
29	HS+ X Oh43+	26.08	25.53	25.45	25.7	26.21
17	Hi38bt X Hi27bt	12.75	12.56	13.08	12.8	
5	Hi27bt X B37bt	17.42	16.49	17.28	17.1	
6	Hi27bt X Oh43bt	18.51	18.03	18.62	18.4	
30	HSbt X Hi27bt	12.24	12.44	12.08	12.3	
18	Hi38bt X B37bt	17.04	16.09	16.66	16.6	
19	Hi38bt X Oh43bt	16.28	16.47	17.51	16.8	
20	Hi38bt X HSbt	13.17	13.22	13.52	13.3	
25	Oh43bt X B37bt	18.73	19.64	18.08	18.8	
31	HSbt X B37bt	19.7	18.36	17.27	18.4	*bt*
32	HSbt X Oh43bt	20.81	19.82	18.14	19.6	16.4
7	Hi27sh2 X B37sh2	11.22	10.84	11.24	11.1	
8	Hi27sh2 X Oh43sh2	12.24	11.72	10.53	11.5	
9	Hi27sh2 X HSsh2	12.68	13.21	12.97	13.0	
26	Oh43sh2 X B37sh2	10.31	10.65	11.5	10.8	
33	HSsh2 X B37sh2	12.35	12.35	12.4	12.4	*sh2*
34	HSsh2 X Oh43sh2	13.53	13.27	13.08	13.3	12.01
10	Hi27su X Hi38su	21.22	20.49	21.17	21.0	
11	Hi27su X B37su	21.07	21.63	22.53	21.7	
12	Hi27su X Oh43su	20.77	20.85	20.61	20.7	
13	Hi27su X HSsu	20.25	20.01	19.38	19.9	
21	Hi38su X B37su	25.74	24.51	22.28	24.2	
22	Hi38su X Oh43su	21.47	21.61	21.88	21.7	
23	Hi38su X HSsu	22.93	21.5	21.46	22.0	
27	Oh43su X B37su	19.41	19.27	19.85	19.5	
35	HSsu X B37su	22.14	24.3	22.49	23.0	*su*
36	HSsu X Oh43su	20.39	20.45	21.08	20.6	21.43
	@MAX - @MIN	18.25	17.21	18.1	17.3	
	Average	19.98	19.73	19.66	**19.8**	
	CV%	0.28	0.27	0.27	0.27	

Appendix 13.

The average data* of sensory test for the 26 varieties. (bt1, sh2 and su1 only) (1 - 9 Scale)**

Plot	Entry	Flavor		Sweetness		Tenderness		Crispness	
		Fresh	Cooke	Fresh	Cooke	Fresh	Cooke	Fresh	Cooked
5	Hi27bt X B37bt	5.61	5.39	5.22	6.06	7.72	6.72	4.72	6.56
6	Hi27bt X Oh43bt	5.89	5.44	5.39	5.72	6.89	5.67	4.78	6.17
17	Hi38bt X Hi27bt	3.83	4.50	2.94	4.56	5.56	5.56	4.83	5.67
18	Hi38bt X B37bt	5.94	5.78	5.94	6.11	7.67	6.28	4.56	6.17
19	Hi38bt X Oh43bt	6.39	5.28	6.17	5.61	6.72	5.28	4.67	5.78
20	Hi38bt X HSbt	2.44	2.17	1.67	1.78	2.61	2.11	3.72	4.89
25	Oh43bt X B37bt	6.61	6.17	5.67	6.61	8.06	7.00	4.89	6.17
30	HSbt X Hi27bt	3.00	2.56	2.22	2.44	4.61	3.00	4.17	4.33
31	HSbt X B37bt	5.94	5.56	5.67	6.83	6.89	6.28	4.61	6.67
32	HSbt X Oh43bt	5.22	6.61	7.06	6.83	6.67	5.89	4.39	6.56
	Average	**5.09**	**4.94**	**4.79**	**5.26**	**6.34**	**5.38**	**4.53**	**5.89**
7	Hi27sh2 X B37sh2	5.39	4.17	4.17	3.89	7.61	5.94	4.83	5.72
8	Hi27sh2 X Oh43sh2	5.22	4.61	4.00	4.28	7.67	5.94	4.67	5.67
9	Hi27sh2 X HSsh2	4.94	4.33	4.11	4.28	6.17	5.17	4.50	6.00
26	Oh43sh2 X B37sh2	4.89	4.33	3.61	3.67	7.50	5.56	3.94	5.22
33	HSsh2 X B37sh2	4.50	3.94	2.72	2.78	5.17	5.56	4.39	5.00
34	HSsh2 X Oh43sh2	5.28	4.11	3.50	3.89	6.56	5.06	4.06	5.22
	Average	**5.04**	**4.25**	**3.69**	**3.80**	**6.78**	**5.54**	**4.40**	**5.47**
10	Hi27su X Hi38su	5.67	6.11	6.78	7.44	5.33	3.44	6.78	7.33
11	Hi27su X B37su	6.89	6.22	7.56	7.44	7.78	6.00	7.33	7.56
12	Hi27su X Oh43su	5.67	5.33	6.56	6.44	6.22	4.67	6.33	7.00
13	Hi27su X HSsu	6.44	7.11	7.78	7.67	4.78	4.56	6.67	7.44
21	Hi38su X B37su	6.33	6.44	7.00	7.22	6.89	5.89	7.00	7.44
22	Hi38su X Oh43su	4.78	4.00	6.33	5.89	5.00	4.11	6.56	7.78
23	Hi38su X HSsu	3.89	4.44	5.44	7.22	3.00	2.78	7.22	6.89
27	Oh43su X B37su	6.33	6.33	6.56	7.33	7.89	6.89	6.33	6.78
35	HSsu X B37su	7.11	5.67	7.56	6.78	7.44	6.67	7.22	7.56
36	HSsu X Oh43su	6.33	5.56	7.11	7.00	5.89	5.00	6.44	7.11
	Average	**5.94**	**5.72**	**6.87**	**7.04**	**6.02**	**5.00**	**6.79**	**7.29**

* Each data is an average of 3 samples from two biting tests, within each biting test, 3 panels' biting data from the same ear were averaged.

** For the 1-9 scale used here, 1 represented the best and 9 was the worst.

Appendix 14.

Tenderness data* of sensory test for the 26 varieties (bt1, sh2 and su1 only) (1 - 9 Scale)**

Plot	Entry	Tenderness Fresh Sam1	Sam2	Sam3	Avg	Tenderness Cooked Sam1	Sam2	Sam3	Avg
5	Hi27bt X B37bt	8.33	7.33	7.50		6.33	6.83	7.00	
6	Hi27bt X Oh43bt	6.83	6.83	7.00		5.83	5.50	5.67	
17	Hi38bt X Hi27bt	5.83	5.50	5.33		5.00	6.17	5.50	
18	Hi38bt X B37bt	7.33	7.83	7.83		5.83	6.00	7.00	
19	Hi38bt X Oh43bt	7.00	6.83	6.33		5.50	4.33	6.00	
20	Hi38bt X HSbt	2.67	2.67	2.50		2.83	1.67	1.83	
25	Oh43bt X B37bt	8.17	8.00	8.00		6.50	7.17	7.33	
30	HSbt X Hi27bt	4.83	3.83	5.17		2.50	3.33	3.17	
31	HSbt X B37bt	7.33	7.83	5.50	*bt*	6.33	6.00	6.50	*bt*
32	HSbt X Oh43bt	6.83	6.33	6.83	6.34	5.83	5.50	6.33	5.38
7	Hi27sh2 X B37sh2	7.50	7.50	7.83		5.83	6.00	6.00	
8	Hi27sh2 X Oh43sh2	8.17	7.67	7.17		5.50	6.00	6.33	
9	Hi27sh2 X HSsh2	5.83	6.33	6.33		5.50	4.83	5.17	
26	Oh43sh2 X B37sh2	7.67	7.50	7.33		5.33	6.00	5.33	
33	HSsh2 X B37sh2	6.00	5.33	4.17	*sh2*	5.50	5.33	5.83	*sh2*
34	HSsh2 X Oh43sh2	6.50	6.33	6.83	6.78	4.67	5.17	5.33	5.54
10	Hi27su X Hi38su	5.67	5.33	5.00		2.67	4.00	3.67	
11	Hi27su X B37su	8.00	7.67	7.67		5.67	5.67	6.67	
12	Hi27su X Oh43su	6.33	6.33	6.00		4.33	5.33	4.33	
13	Hi27su X HSsu	6.00	4.00	4.33		5.33	4.00	4.33	
21	Hi38su X B37su	6.67	7.33	6.67		5.33	6.00	6.33	
22	Hi38su X Oh43su	5.00	4.67	5.33		5.00	4.00	3.33	
23	Hi38su X HSsu	3.33	2.67	3.00		2.67	3.00	2.67	
27	Oh43su X B37su	7.33	7.67	8.67		6.67	7.00	7.00	
35	HSsu X B37su	7.33	7.33	7.67	*su*	6.67	7.00	6.33	*su*
36	HSsu X Oh43su	6.67	5.33	5.67	6.02	5.00	5.67	4.33	5.00
	@MAX - @MIN	5.67	5.33	6.17		4.17	5.50	5.50	
	Average	6.51	6.23	6.22	**6.32**	5.16	5.29	5.36	**5.27**
	CV%	0.21	0.26	0.25		0.24	0.25	0.28	

* Each datum is an average of 3 samples from two biting tests. Within each biting test, 3 panelist's biting data from the same ear were averaged.

** For the 1-9 scale used here, 1 represented the best and 9 was the worst.

Appendix 15.

Sweetness data* of sensory test for the 26 varieties (bt1, sh2 and su1 only) (1 - 9 Scale)**

Plot	Entry	Sweetness Fresh Sam1	Sam2	Sam3	Avg	Sweetness Cooked Sam1	Sam2	Sam3	Avg
5	Hi27bt X B37bt	4.83	4.83	6.00		6.17	5.67	6.33	
6	Hi27bt X Oh43bt	4.50	5.50	6.17		5.33	5.50	6.33	
17	Hi38bt X Hi27bt	3.17	2.83	2.83		4.50	4.33	4.83	
18	Hi38bt X B37bt	5.17	6.17	6.50		6.50	6.00	5.83	
19	Hi38bt X Oh43bt	6.00	6.50	6.00		5.33	5.50	6.00	
20	Hi38bt X HSbt	2.17	1.67	1.17		2.00	1.83	1.50	
25	Oh43bt X B37bt	5.17	6.33	5.50		6.17	6.50	7.17	
30	HSbt X Hi27bt	2.33	1.83	2.50		2.00	2.50	2.83	
31	HSbt X B37bt	5.50	5.67	5.83	*bt*	6.17	7.33	7.00	*bt*
32	HSbt X Oh43bt	6.67	7.00	7.50	4.79	6.83	6.33	7.33	5.26
7	Hi27sh2 X B37sh2	4.33	4.33	3.83		3.83	3.67	4.17	
8	Hi27sh2 X Oh43sh2	4.17	3.67	4.17		4.00	4.50	4.33	
9	Hi27sh2 X HSsh2	4.17	4.33	3.83		3.67	4.17	5.00	
26	Oh43sh2 X B37sh2	3.67	3.50	3.67		3.17	4.50	3.33	
33	HSsh2 X B37sh2	3.33	2.67	2.17	*sh2*	2.33	3.17	2.83	*sh2*
34	HSsh2 X Oh43sh2	3.67	3.33	3.50	3.69	3.67	3.83	4.17	3.80
10	Hi27su X Hi38su	6.33	6.67	7.33		7.33	8.00	7.00	
11	Hi27su X B37su	7.33	7.33	8.00		7.33	7.33	7.67	
12	Hi27su X Oh43su	6.33	7.00	6.33		6.00	6.67	6.67	
13	Hi27su X HSsu	7.67	7.67	8.00		7.67	7.67	7.67	
21	Hi38su X B37su	6.67	7.33	7.00		7.33	7.00	7.33	
22	Hi38su X Oh43su	6.00	6.67	6.33		5.67	6.00	6.00	
23	Hi38su X HSsu	5.33	5.33	5.67		7.33	7.33	7.00	
27	Oh43su X B37su	6.33	6.33	7.00		7.33	7.00	7.67	
35	HSsu X B37su	7.67	7.33	7.67	*su*	6.33	7.00	7.00	*su*
36	HSsu X Oh43su	7.33	6.67	7.33	6.87	7.00	7.33	6.67	7.04
	@MAX - @MIN	5.50	6.00	6.83		5.67	6.17	6.17	
	Average	5.22	5.33	5.46	**5.34**	5.42	5.64	5.76	**5.61**
	CV%	0.31	0.34	0.36		0.33	0.30	0.30	

* Each datum is an average of 3 samples from two biting tests. Within each biting test, 3 panelist's biting data from the same ear were averaged.

** For the 1-9 scale used here, 1 represented the best and 9 was the worst.

Appendix 16.

Flavor data* of sensory test for the 26 varieties (bt1, sh2 and su1 only) (1 - 9 Scale)**

Plot	Entry	Flavor Fresh Sam1	Sam2	Sam3	Avg	Flavor Cooked Sam1	Sam2	Sam3	Avg
5	Hi27bt X B37bt	5.67	5.00	6.17		5.67	5.00	5.50	
6	Hi27bt X Oh43bt	5.33	5.50	6.83		5.33	5.50	5.50	
17	Hi38bt X Hi27bt	3.67	3.83	4.00		4.33	4.00	5.17	
18	Hi38bt X B37bt	5.33	6.33	6.17		5.67	5.50	6.17	
19	Hi38bt X Oh43bt	5.83	6.50	6.83		4.83	5.33	5.67	
20	Hi38bt X HSbt	2.67	2.17	2.50		2.50	2.00	2.00	
25	Oh43bt X B37bt	6.00	7.00	6.83		5.50	6.33	6.67	
30	HSbt X Hi27bt	3.50	2.50	3.00		2.17	2.67	2.83	
31	HSbt X B37bt	5.83	5.67	6.33	*bt*	5.00	5.83	5.83	*bt*
32	HSbt X Oh43bt	4.67	5.50	5.50	5.09	6.33	6.50	7.00	4.94
7	Hi27sh2 X B37sh2	5.00	5.67	5.50		4.33	4.00	4.17	
8	Hi27sh2 X Oh43sh2	5.00	5.33	5.33		4.50	4.67	4.67	
9	Hi27sh2 X HSsh2	4.67	4.83	5.33		4.33	4.17	4.50	
26	Oh43sh2 X B37sh2	4.67	5.17	4.83		4.17	4.83	4.00	
33	HSsh2 X B37sh2	4.83	4.83	3.83	*sh2*	3.83	4.00	4.00	*sh2*
34	HSsh2 X Oh43sh2	5.50	5.00	5.33	5.04	3.83	4.33	4.17	4.25
10	Hi27su X Hi38su	5.00	6.00	6.00		5.33	6.33	6.67	
11	Hi27su X B37su	6.67	6.67	7.33		6.00	6.00	6.67	
12	Hi27su X Oh43su	6.33	5.33	5.33		5.00	5.00	6.00	
13	Hi27su X HSsu	5.67	6.67	7.00		6.67	7.67	7.00	
21	Hi38su X B37su	6.00	6.33	6.67		6.33	6.33	6.67	
22	Hi38su X Oh43su	4.67	4.67	5.00		3.33	4.33	4.33	
23	Hi38su X HSsu	3.67	4.00	4.00		3.67	5.00	4.67	
27	Oh43su X B37su	5.67	6.33	7.00		5.67	6.33	7.00	
35	HSsu X B37su	6.67	7.33	7.33	*su*	5.00	5.67	6.33	*su*
36	HSsu X Oh43su	7.00	5.67	6.33	5.94	5.67	5.67	5.33	5.72
	@MAX - @MIN	4.33	5.17	4.83		4.50	5.67	5.00	
	Average	5.21	5.38	5.63	**5.41**	4.81	5.12	5.33	5.08
	CV%	0.20	0.23	0.23		0.24	0.24	0.25	

* Each datum is an average of 3 samples from two biting tests. Within each biting test, 3 panelist's biting data from the same ear were averaged.

** For the 1-9 scale used here, 1 represented the best and 9 was the worst.

Appendix 17.

Crispness data* of sensory test for the 26 varieties (bt1, sh2 and su1 only) (1 - 9 Scale)**

Plot	Entry	Crispness Fresh Sam1	Sam2	Sam3	Avg	Crispness Cooked Sam1	Sam2	Sam3	Avg
5	Hi27bt X B37bt	4.67	4.67	4.83		6.67	6.17	6.83	
6	Hi27bt X Oh43bt	4.50	4.83	5.00		5.67	6.67	6.17	
17	Hi38bt X Hi27bt	5.00	4.83	4.67		5.17	5.50	6.33	
18	Hi38bt X B37bt	3.83	4.67	5.17		6.00	6.33	6.17	
19	Hi38bt X Oh43bt	4.67	4.50	4.83		5.67	6.00	5.67	
20	Hi38bt X HSbt	3.83	3.83	3.50		5.17	4.67	4.83	
25	Oh43bt X B37bt	4.67	4.83	5.17		5.50	6.33	6.67	
30	HSbt X Hi27bt	4.33	4.17	4.00		4.17	4.33	4.50	
31	HSbt X B37bt	4.67	4.33	4.83	*bt*	6.83	6.83	6.33	*bt*
32	HSbt X Oh43bt	4.17	4.33	4.67	4.53	6.50	6.33	6.83	5.89
7	Hi27sh2 X B37sh2	4.83	4.83	4.83		6.33	5.67	5.17	
8	Hi27sh2 X Oh43sh2	4.50	4.83	4.67		5.83	5.33	5.83	
9	Hi27sh2 X HSsh2	4.33	4.50	4.67		6.00	5.67	6.33	
26	Oh43sh2 X B37sh2	4.00	3.83	4.00		5.33	5.17	5.17	
33	HSsh2 X B37sh2	4.17	4.50	4.50	*sh2*	4.83	4.83	5.33	*sh2*
34	HSsh2 X Oh43sh2	3.83	4.00	4.33	4.40	5.00	5.50	5.17	5.47
10	Hi27su X Hi38su	6.33	6.67	7.33		7.00	7.67	7.33	
11	Hi27su X B37su	7.00	7.33	7.67		7.33	7.33	8.00	
12	Hi27su X Oh43su	6.33	6.00	6.67		7.33	7.00	6.67	
13	Hi27su X HSsu	6.33	6.67	7.00		7.67	7.33	7.33	
21	Hi38su X B37su	7.00	7.00	7.00		7.67	7.33	7.33	
22	Hi38su X Oh43su	6.67	6.33	6.67		7.67	8.00	7.67	
23	Hi38su X HSsu	7.00	7.00	7.67		6.67	7.00	7.00	
27	Oh43su X B37su	6.00	6.33	6.67		7.00	6.33	7.00	
35	HSsu X B37su	7.00	7.67	7.00	*su*	7.67	7.33	7.67	*su*
36	HSsu X Oh43su	6.67	6.33	6.33	6.79	7.33	7.00	7.00	7.29
	@MAX - @MIN	3.17	3.83	4.17		3.50	3.67	3.50	
	Average	5.24	5.34	5.53	**5.37**	6.31	6.29	6.40	**6.33**
	CV%	0.22	0.22	0.23		0.16	0.16	0.15	

* Each datum is an average of 3 samples from two biting tests. Within each biting test, 3 panelist's biting data from the same ear were averaged.

** For the 1-9 scale used here, 1 represented the best and 9 was the worst.

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